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Allium cameleon extract protects against Aluminum chloride-induced reproductive toxicity in male rat (*Rattus norvegicus*) probably by attenuating oxidative damages, inflammatory effect and increasing sexual hormone

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Abstract

Objective: The aim of this study is to investigate effects of *Allium cameleon* Cronquist (1981) (Liliaceae) a bulb belonging to the garlic family, on reproductive toxicity induced by aluminum chloride, known as a medicinal plant used for its aphrodisiac activities in Cameroonian folk medicine. The current investigation was carried out to evaluate the protective potentials of *Allium cameleon* extracts and study its possible mechanisms by which this could be accomplished

Methods: The behavioral and biochemical tests were used to detect the protective properties. The conceivable mechanisms of action of the extracts were investigated through determination of sexual hormones, nitric oxide, cGMP, anti-inflammatory and antioxidant activities in male rats treated with aluminum chloride and plant extract.

Results: Aluminum chloride caused reproductive toxicity by decreasing sexual excitement, semen quality, and the concentrations of testosterone, FSH and LH. Levels of endogenous antioxidant enzymes (CAT, SOD, and GSH) were worsened. Inflammatory parameters were improved. *Allium cameleon* thwarted all these parameters. Penile nitric oxide and cGMP levels were boosted. Significant stimulation in sexual behavior, elevated spermatic production, raised of viability, morphology, count and sperm mobility were also observed. Treated animals exhibited elevated levels of endogenous antioxidant enzymes and inflammatory cytokines were decreased.

Conclusion: These discoveries suggest that *Allium cameleon* protects animals against reproductive toxicity that might be involve in the attenuation of oxidative and inflammatory damage, but also in the increase of sexual hormones production and NO/cGMP cascade.

Keywords: *Allium Cameleon*; Reproductive Toxicity; Aluminum Chloride; Sexual Hormones; Folk Medicine.

1. Introduction

Infertility represents a real public health problem and is considered a pathology in its own right. Indeed, around 84% of couples in general are expected to conceive within a year and around 92% within two years of sexual intercourse [1]. Today, around 15% of couples have difficulty conceiving. In 50% of cases, the cause is due to a female factor, in 20-30%

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of cases to a male factor and in the remaining 20-30% it is a combination of female and male factors [2]. The World Health Organization (WHO) defines infertility as the inability of a couple to procreate or to carry a pregnancy to term after at least one year of regular, unprotected sexual intercourse [3]. The repeated inability of the male sexual organs and penile erectile factors to function effectively, or a disorder that interferes with his complete sexual response cycle, is referred to as male sexual dysfunction [4]. Male sexual dysfunction is common worldwide in men of all ages, ethnic origins and cultural backgrounds [5]. Male sexual dysfunctions take different forms, such as desire disorders, orgasm disorders, erectile dysfunctions, ejaculation disorders and detumescence failure. Erectile dysfunction and premature ejaculation are the two most frequent complaints of male patients with sexual disorders [6], [7]. They are of varied etiology and include personal lifestyles, androgen deficiency, aging, psychological disorders, side effects of certain drugs and anti-androgens [8]. Metals can have serious effects on the male reproductive system, either directly or indirectly [9]. Metals have been shown to affect spermatogenesis in rodents and humans, leading to low sperm counts, abnormal sperm morphology and poor sperm quality [10], [11]. Although aluminum is present in trace amounts in biological material, it does not appear to be an essential element and is generally considered to have adverse health effects [12], [13]. This study was undertaken to assess the effects of *Allium cameleon* aqueous extract on behaviors and sexual and hematological parameters of rats pre-treated with aluminum chloride and to propose its mechanisms of action.

2. Material and methods

The materials and methods used in the present work are those described in our previous work with some modifications [14]. As a reminder, we describe the said materials and methods in the following paragraphs.

2.1. Plant material

The aerial *Allium cameleon* parts used in these experiments were harvested between March and April 2018 at Maroua, in the Far-North Region of Cameroon, without endangering the protected species. The collected plant was identified by botanist of the University of Maroua and confirmed by Cameroon National herbarium where a voucher specimen was deposited with voucher number 67481HNC.

2.2. Preparation of *Allium cameleon* aqueous extracts

The aerial *Allium cameleon* parts were ground, then, the resulting powder (100 g) was macerated in 1000 mL of distilled water for 1 hour. The obtained mixture was boiled for 20 minutes before filtration of the supernatant through the Whatman No 1 paper. The subsequent aqueous extract (decoction) was then administered orally to male and female rats in a volume of 10 mL/kg. The decoctions of *Allium cameleon* were prepared daily according to Traditional Healers' instructions. In another set of experiment the decoction was concentrated using a rotary vacuum evaporator under reduced pressure at 50°C, and from this procedure the extraction yield was calculated. The stock solution of *Allium cameleon* extract (decoction at 20 mg/mL) was diluted in distilled water, to obtained two less concentrated solutions of 15 and 10 mg/mL.

2.3. Assessment of the sexual behavior of male rats

Seventy-two rats (thirty-six sexually active male (220–250 g) and thirty-six females (150–160 g) were used for this work. Each animal was kept in separate cages under a reversed 12 hours light/dark cycle. Standard rodent food and tap water were available unlimitedly. Rats were randomly divided into six groups. Each group contained 6 rats and distribute as follows: Group I - the normal control - was treated with vehicle (distilled water) 10ml/kg;

- Group II- is treated with aluminum chloride (AlCl3 (100 mg/kg/d));
- Group III- receives AlCl3 (100 mg/ kg/d) and *Allium cameleon* 200 mg/kg;
- Group IV- receives *Allium cameleon* (200 mg/kg/d);
- Group V- treated with AlCl3 (100 mg/kg/d) and sildenafil citrate (60 mg/kg/d);
- Group VI- received sildenafil citrate (60 mg/kg/d).

Extracts were administered as single daily dose through the oral route for 30 days. The methods adopted for sexual behavior testing of male rats in this study were described previously by Yakubu *et al*. [8]. Only female rats on estrus were allowed to mate with males. The females tested were artificially made estrus by administration of estradiol benzoate 10µg/100 g and progesterone 0.5 mg/100g bw subcutaneously 48 hours and 4 hours respectively before the experiment [8].

During the study, male rats were placed individually in the experimental apparatus for sexual behavior. After 10 minutes of acclimation; sexually receptive females were introduced into the cage in a 1:1 ratio. Thirty (30) minutes were given to each pair and the following pre-copulatory and copulatory behaviors were assessed:

- The time from the introducing of a female rat into the cage of the male until the first mount (mount latency (ML));
- The time from the introducing of a female rat into the cage until the first intromission by the male (intromission latency (IL));
- The number of mounts before ejaculation (mount frequency (MF))
- The number of intromissions before ejaculation (intromission frequency (IF))
- The time from the first intromission of a series until the ejaculation (ejaculation latency (EL))
- The time from ejaculation until the first intromission of the following series (post ejaculatory interval (PEI)).
- The number of ejaculations in a copulatory series (Ejaculation frequency (EF))
- The number of bends to lick its penis (Erection frequency (ErF))

Sexual behaviors experiments were performed 3 hours after the last dose of the extract was administered.

2.4. Sexual organs weight, sperm count, motility, morphology and viability

At the end of the trial, animals were sacrificed by severing the jugular vein. Testes, seminal vesicles, prostates, epididymis, and vas deferens were carefully removed, discarded of adipose tissue, then the three first were blot-dried, and weighed. Paired organs were weighed individually, and weights summed up to obtain the paired weight of the organs. Immediately after sacrifice, epididymis of each animal was removed and immersed in 10 ml of warm 0.9% NaCl solution (40 °C), while spermatozoa were obtained from epididymis following the prescriptions Sharma *et al*. [15]. To assess the motility, 20 µl was used following the scale basis as described by Mohammed and Engidawork [16]. Sperm viability expressed as percentage of swollen semen was analyzed using hypo-osmotic swelling test [17], whereas the morphology, which is the percentage of normal semen, was analyzed using Eosin/Nigrosin test. Five microliters of semen were mixed with 5 µl of Eosin/Nigrosin solution, and morphological defects of head, mid-piece, tail, and the proportions of cells affected were evaluated. For each of both parameters (viability and morphology), a total of 200 spermatozoa were counted in at least five different microscope fields, following the protocol described by Revell and Mrod [18]. The sperm count was analyzed by homogenizing the epididymis in 5 ml of normal saline and then counted using a haemocytometer.

2.5. Serum hormones essays

After administrating of the crude extract for 30 days of experimentation, blood samples were collected after section of the jugular vein and put into a flask tube without anticoagulant, allowed to stand and centrifuged at 3000 rpm for 15 min at 4°C to collect the sera for testosterone, FSH and LH determination. Plasmatic testosterone concentration was evaluated using a standard kit (Accubind, Monobind Inc. Lake Forest, USA) according to the manufacturer's instructions. The concentration of Follicle Stimulating Hormone (FSH) was estimated using the FSH immunoradiometric assay (FSH IRMA) with kits according to the manufacturer's protocol. The concentration of luteinizing hormone (LH) was determined by radioimmunoassay (HLH31-K01) with kits according to the manufacturer's protocol.

2.6. Nitric oxide and cGMP essays

2.6.1. Determination of nitric oxide level in the penile tissues

After experimental period, animals were sacrificed. Then, the penile tissues were quickly removed and washed in cold saline solution, blotted on filter papers to remove adhering blood, and homogenized in 100 mM sodium phosphate, pH 7.4. The homogenates were centrifuged at 10,000 g for 20 min at 4°C. Nitric oxide level in penile tissue homogenate was estimated in a medium containing 400 mL of 2% vanadium chloride (VCl3) in 5% HCl, 200 ml of 0.1% N-(l-naphthyl) ethylenediamine dihydrochloride, 200 mL of 2% sulphanilamide (in 5% HCl). After incubation at 37°C for 60 min, nitrite levels, which correspond to an estimative level of NO, were determined spectrophotometrically at 540 nm, based on the reduction of nitrate to nitrite by VCl3 [19]. Penile tissue nitrite level was expressed as micromole of NO per gram of tissue.

2.6.2. Determination of cGMP level in the penile tissues

An ELISA kit (MBS007871) with 96 wells was used to quantify the cGMP levels. Selected plate wells contained known concentrations of cGMP (50, 10, 2, 0.4, 0.08 pmol/mL) prepared by serial dilutions to make the standard curve. The frozen tissues were ground into very fine pieces and dissolved in 0.1 mol/L HCl. An aliquot of the supernatant containing

cGMP from the penile tissues was placed in the wells after centrifugation. Both the standard and the test wells were treated with a yellow antibody, and after incubation and washing, the plate was read by a microplate reader at 405 nm; cGMP levels were calculated using the standard curve and expressed as μmol/mg protein.

2.7. Oxidative stress level determination

2.7.1. Superoxide dismutase activities determination.

Total superoxide dismutase (SOD) activity was evaluated with SOD detection kit (RANSOD kit produced by RANDOX Company, Northern Ireland Antrim, UK) according to the manufacturer's instructions. This method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD causes 50% inhibition in the rate of reduction of INT under the conditions of the assay. SOD levels were recorded at 505 nm and through a standard curve, and expressed as unit per gram of tissue protein (U/g protein).

2.7.2. Catalase activities measurement.

Tissue catalase (CAT) activity was determined spectrophotometrically by monitoring the decomposition of H_2O_2 using the protocol proposed by Aebi [20]. Briefly, 0.5 mL of

30 mmol/L H_2O_2 solution was mixed in 50 mmol/L phosphate buffer (pH 7.0), 1 mL of 1:10 diluted testis supernatant, was added and the depletion of H_2O_2 was followed spectrophotometrically at 240 nm for 2 minutes at 25°C. The molar extinction coefficient was 43.6 L/mol per cm for H_2O_2. CAT activity was expressed as the unit that is defined as mmol H2O2 consumed/min per g tissue protein.

2.7.3. Determination of Lipid Peroxidation

To determine malondialdehyde (MDA) level in serum tissue, 200 mL of phosphoric acid 85% and 25 mL of thiobarbituric acid (TBA) were added to the 2 micro tubes containing 0.2 mL mitochondrial fractions (0.5 mg protein/mL) and then placed in a boiling water bath for 30 minutes. The tubes were shifted to an ice-bath to decrease temperature for 3 minutes. The solution was centrifuged at 6000 rpm for 6 minutes. Fatty acids reacted with TBA to produce a purple complex that can be determined by the spectrophotometer. Finally, the supernatant absorption was measured at 535 nm. The level of tissue MDA was expressed as mol/g of protein. The method was calibrated with tetramethoxypropane and ethanol 40% standard solutions [21].

2.7.4. Glutathione (GSH) Assay

The method described by Beutler *et al.* [22] was used for the determination of GSH level. The homogenized tissue was mixed with 1.5 mL EDTA reagent and then the 1.5 mL trichloroacetic acid (TCA) solution (TAC 10%: first, 3.722 g EDTA dissolved in 500 ml DW, then, 5 g TCA dissolved in 50 mL first solution) was added. The tube was centrifuged at 3500 rpm for 15 minutes. Then 1 mL of supernatant was mixed with 2.5 mL Tris buffer (pH 8.9) and 500 mL TNB reagent (0.245 g TNB dissolved in 250 mL phosphate buffer). The yellow color solution so developed were read at 412 nm on a spectrophotometer. GSH content was expressed as moL/g of protein. The GSH standards were prepared by dissolving 0.0115 g GSH in 100 mL of distilled water.

2.8. Determination of inflammation markers

Cerebral markers were determined by the enzyme-linked immunosorbent assay (ELISA) using Quantikine kits (France).

2.8.1. Determination of interleukin 1-β levels

Fifty microliters of the diluted standard solution and the various treatments were introduced into the wells. Following the addition of 200 μL of wash solution, each well was washed 5 times until all liquid was completely removed. After the fifth wash, 50 μL of IL1-β antibody was added to each well. The plate was then incubated at room temperature for 2 h. After one to two hours, the mixture was removed by aspiration, and 200 μL of wash solution was introduced into each well, then poured in. On completion of washing, 100 μL of streptavidin peroxidase solution was then introduced into each well, and left to incubate at room temperature for 30 min. After 30 min, the solution was poured off and washed by introducing 200 μL of wash solution into each well. This wash was carried out five times. Next, 50 μL of chromogen (TMB) was added to the plate, covered with aluminum foil and incubated for a further 25 min at room temperature. At the end, 50 μL of the stop solution was added to each well, then the plate was read by a spectrophotometer at 450 nm.

2.8.2. Determination of tumor necrosis factor alpha levels

Standards, blank and samples are pipetted into wells containing TNF-α-specific antibodies. TNF-α present in the various solutions will bind to the immobilized antibodies. After washing, to remove unbound TNF-α, a specific enzyme conjugated to a secondary polyclonal antibody is added to the wells. After washing to remove the unbound antibody-enzyme complex, a substrate is added to the wells. The resulting enzymatic reaction leads to a color change from blue to yellow, then the reaction is stopped by a stop solution. Absorbance is read on a spectrophotometer at 540 nm. One hundred microliters of each concentration of the diluted standard solution and the various treatments were introduced into the wells. The plate containing the wells was protected by aluminum foil and incubated for 2 h at 37°C. The solution was then poured off, and 100 µL of TNF-alpha antibody was added to each well. The plate was then incubated at room temperature for one hour. After one hour, the mixture was removed and 300 µL of wash solution was poured into each well. At the end of the third wash, the wells were cleaned with tissue. The avidin solution (100 µL) was then introduced into each well, and left to incubate at room temperature for 30 min. After 30 min, the solution was poured off and washed by introducing 350 µL of wash solution into each well. This wash was carried out five times and 100 µL of tetramethylbenzidine (TMB) was then added to the plate, covered with aluminum foil and incubated for a further 25 min at room temperature. Fifty microliters of stop solution were added to each well, then the plate was read by a spectrophotometer at 450 nm.

2.8.3. Determination of interleukin 6 levels

One hundred microliters of each concentration of the diluted standard solution and the various treatments were introduced into the wells. The plate containing the wells was protected by aluminum foil and incubated for 2 h at 37°C. The solution was then poured in. One hundred microliters of IL-6 antibody were added to each well. The plate was then incubated at room temperature for one hour. After one hour, the mixture was removed and 300 µL of wash solution was poured into each well. At the end of the third wash, the wells were cleaned with tissue. One hundred microliters of avidin solution were then introduced into each well, and left to incubate at room temperature for 30 min. After 30 min, the solution was poured off and washed by introducing 350 µL of wash solution into each well. This wash was carried out five times, and 100 µL of tetramethylbenzidine (TMB) was then added to the plate, covered with aluminum foil and incubated for a further 25 min at room temperature under gentle stirring. 50 µL of stop solution was added to each well, and the plate was then read by a spectrophotometer at 450 nm

2.9. Statistical analysis

Data were expressed as mean ± SEM (n=6). Difference between means of various treatment groups with the negative control group was determined by analysis of variance and Tukey's post-test using XLSTAT 2018 version 20.1.49320. Value of $P < 0.05$ was considered significant.

3. Results

3.1. Effects of *Allium cameleon* on Aluminum chloride-induced alteration of sexual behavior in male rats

3.1.1. Effects of Allium cameleon on altered latency of mount, intromission and ejaculation induced by $AlCl_3$

Table 1 shows the effects of aluminum chloride ($AlCl_3$) on ejaculation, mount and intromission latencies. The ANOVA shows that the different groups presented significant variability for all the parameters studied [$F(5, 30) = 114, 34$, $p<0.001$]. This table shows that $AlCl_3$ significantly increases mount latency ($p<0.001$), ranging from 98.17±3.76 seconds for the distilled water group to 182.00±5.55 seconds for the 100 mg/kg $AlCl_3$ group ($p<0.001$). Simultaneous administration of $AlCl_3$ and plant extract at 200 mg/kg bring back this time to 98.50±4.37 seconds ($p<0.001$). Plant extract alone, at a dose of 200 mg/kg, significantly reduced this time to 47.50±3.62 ($p<0.001$). Similarly, a significant lengthening ($p<0.001$) of intromission latency was observed in animals treated with $AlCl_3$. This time ranged from 111.33±2.80 seconds for the negative control group, treated with distilled water, to 213.33±7.79 seconds for animals receiving $AlCl_3$ at a dose of 100 mg/kg. Co-administration of 200 mg/kg *Allium cameleon* extract and $AlCl_3$ reduced this time to 121.50±1.87 seconds. For the group treated with plant extract alone, this time decreased to 55.67±1.03 seconds. In contrast to the two previous groups, administration of $AlCl_3$ significantly reduced ejaculation latency ($p<0.001$), which ranged from 212.33±1.51 seconds in animals treated with distilled water to 88.33±4.46 seconds in those given $AlCl_3$. In animals treated with both $AlCl_3$ and *Allium cameleon* leaf extract, this time significantly ($p<0.001$) increased ejaculation latency to 284.17±1.72 seconds. This time increased to 284.17±1.72 in animals treated with the plant at a dose of 200 mg/kg. Interestingly, Sildenafil citrate at 60 mg/ kg acted positively at all doses and all parameters in the study. Also, co-treatment of animals with sildenafil citrate and $AlCl_3$ normalized the various parameters studied. The plant, like the reference molecule, rationalized the various parameters (Table 1).

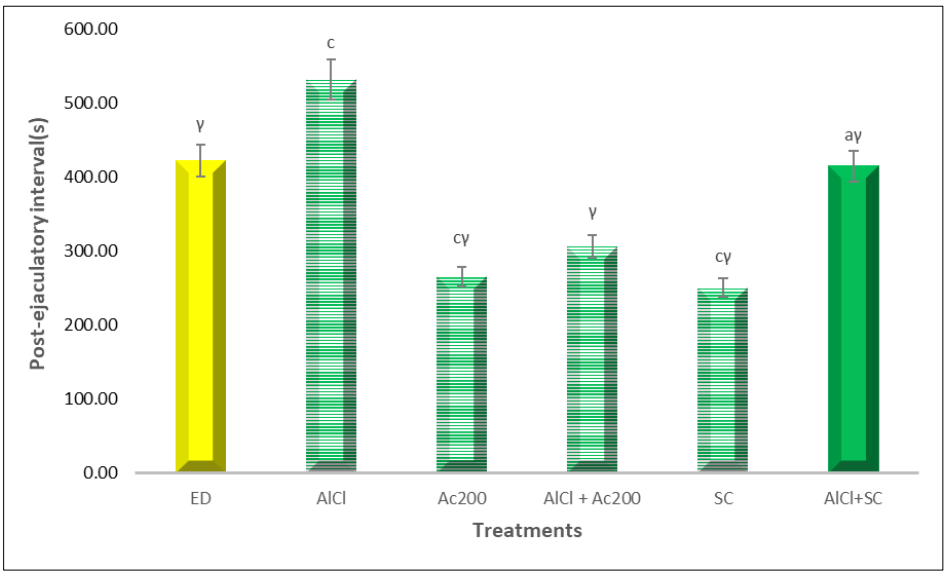
Table 1 Effect of *Allium cameleon* extract on the alteration of mount, intromission and ejaculatory latencies of male rats induced by $AlCl_3$ after daily treatment for 30 days.

Treatments	Sexual behavior parameters (s)		
	Mount Latency	Intromission Latency	Ejaculation Latency
Eau distillée 10 ml/kg	98,17±3,76$^{\gamma}$	111,33±2,80$^{\gamma}$	212,33±1,51
Chlorure d'aluminium 100 mg/kg	182,00±5,55^{c}	213,33±7,79^{c}	88,33±4,46^{c}
Allium cameleon 200 mg/kg	47,50±3,62$^{c\gamma}$	55,67±1,03$^{c\gamma}$	284,17±1,72^{c}
Chlorure d'aluminium 100 mg/kg + *Allium cameleon* 200 mg/kg	98,50±4,37$^{\gamma}$	121,50±1,87$^{\gamma}$	211,50±1,87^{c}
Sildenafil 60 mg/ kg	43,17±1,47$^{c\gamma}$	55,33±1,21$^{c\gamma}$	290,00±4,29^{c}
Sildenafil 60 mg/ kg + Chlorure d'aluminium 100 mg/kg	97,83±4,88$^{\gamma}$	104,83±4,36$^{\gamma}$	214,67±2,88^{c}

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, ^{c}P<0.001, significant difference compared to the distilled water treated group. $^{\gamma}$P<0.001, significant difference compared to Al.^{c}P<0.001, significantly different negative control group.

3.1.2. Effects of Allium cameleon on aluminum chloride-induced alteration of the post-ejaculatory interval.

The data in Figure 1 show the effects of Allium cameleon leaf extract on AlCl3-induced alteration of the post-ejaculatory interval. Overall, significant variability [F (5, 30) = 2234.41, p<0.001] was observed between the different groups studied. The figure shows that AlCl3 significantly (p<0.001) increases the post-ejaculatory interval, which varies from 422.50±4.42 seconds in animals treated with distilled water to 532.33±8.50 seconds in animals treated with aluminum chloride alone. In animals treated simultaneously with plant extract and AlCl3, this time was significantly reduced (p<0.001) to 306.33 ±3.08 seconds. Preparation of the plant alone significantly reduced the post-ejaculatory interval (p< 0.001). This time fell to 265.17±1.72 seconds for the group treated with 200 mg/kg Allium cameleon. Viagra, the reference substance used as a positive control in this study, also reduced the post-ejaculatory interval to 251.00±1.67 seconds. Simultaneous administration of Viagra and AlCl3 reduced the post-ejaculatory interval to 415.50±8.96 seconds. Compared with the control group treated with AlCl3 alone, this reduction is highly significant (p<0.001), figure 1.



Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, aP<0.05, cP<0.001, significantly different compared to distilled water-treated group. γp<0.001, significant difference compared to aluminum chloride-treated group. ED, distilled water; Ac100, 100 mg/kg Allium cameleon aqueous extracts, AlCl: aluminum chloride 100 mg.

Figure 1 Effects of Allium cameleon on post-ejaculatory interval after 30 days of treatment

3.1.3. Effect of Allium cameleon on alteration of mount, intromission, ejaculatory and erection frequencies induced by AlCl3.

Table 2 shows the effects of AlCl3 on mounting, intromission, ejaculation and erection frequencies. The ANOVA shows that all the studied groups present significant variability for all the parameters considered [F (5, 30) = 114, 34, p<0.001]. The table shows also that AlCl3 significantly reduces the frequency of ascent (p<0.001), ranging from 11.83±1.47 for the group treated with distilled water to 3.33±0.82 for the group treated with 100 mg/kg of AlCl3 (p<0.001). Simultaneous administration of AlCl3 and plant extract at a dose of 200 mg/kg restored this number to 9.83±1.47 (p<0.001) compared with the group treated with AlCl3 alone. Plant extract alone, at a dose of 200 mg/kg, significantly increased this time to 19.83±1.83 (p<0.001) compared to both the distilled water and AlCl3 treated groups. Similarly, a significant decrease (p<0.001) in intromission latency was observed in animals treated with AlCl3 compared with those treated with distilled water. This number ranged from 10.17±0.75 for the negative control group, treated with distilled water, to 3.17±0.75 for animals given AlCl3 at a dose of 100 mg/kg. Simultaneous treatment of animals with the 200 mg/kg dose of *Allium cameleon* and AlCl3 reduced this number to 10.00±0.89 (p< 0.001) compared with the group treated with AlCl3 alone. For the group treated with plant extract alone, this number increased to 15.67±1.75 (p< 0.001) compared to animals treated with distilled water and AlCl3. In the same vein as the previous two groups, administration of AlCl3 significantly reduced ejaculation frequency (p< 0.05), which ranged from 1.33±0.52 in animals treated with distilled water to 0.83±0.05 in those given AlCl3. In animals treated with both AlCl3 and *Allium cameleon* extract, the number increased significantly (p<0.05) to 1.83±0.98. This number increased to 2.83±0.75 in animals treated with the plant at a dose of 200 mg/kg (p< 0.001) compared with animals treated with AlCl3. As in previous tests, there was a significant decrease (p<0.001) in erection frequency in animals treated with AlCl3 compared with those treated with distilled water. This number ranged from 6.67±0.82 in the negative control group treated with distilled water to 3.67±0.82 in animals given AlCl3 at a dose of 100 mg/kg. Concomitant administration to animals of the 200 mg/kg dose of *Allium cameleon* powder and AlCl3 reduced this number to 6.17±0.75 (p< 0.001) compared with the group treated with AlCl3. For the group treated with plant extract alone, this number increased to 14.50±1.05 (p< 0.001) compared to animals treated with distilled water and AlCl3. Interestingly, Sildenafil citrate at 60 mg/ kg acted positively at all doses and all parameters in the study. Also, co-treatment of animals with sildenafil citrate and AlCl3 normalized the various parameters studied. The plant, like the reference molecule, rationalized the various parameters (Table 2).

Table 2 Effects of Allium cameleon on aluminum chloride-induced alteration of mount, intromission, ejaculation and erection frequencies of male rats after daily treatment for 30 days.

Treatments	**Sexual behavior frequency**			
	Mount	**Intromission**	**Ejaculation**	**Erection**
Distilled water ml/kg	$11,83±1,47^{\gamma}$	$10,17±0,75^{\gamma}$	$1,33±0,52^{\alpha}$	$6,67±0,82^{\gamma}$
Aluminum chloride 100 mg/kg	$3,33±0,82^{c}$	$3,17±0,75^{c}$	$0,83±0,05^{a}$	$3,67±0,82^{c}$
Allium cameleon 200 mg/kg	$19,83±1,83^{c\gamma}$	$15,67±1,75^{c\gamma}$	$2,83±0,75^{c\gamma}$	$14,50±1,05^{c\gamma}$
Aluminum chloride 100 mg/kg + *Allium cameleon* 200 mg/kg	$9,83±1,47^{\gamma}$	$10,00±0,89^{\gamma}$	$1,83±0,98^{a\beta}$	$6,17±0,75^{\gamma}$
Sildenafil 60 mg/ kg	$22,83±1,17^{c\gamma}$	$16,00±1,26^{c\gamma}$	$2,67±0,52^{a\beta}$	$15,67±1,21^{c\gamma}$
Sildenafil 60 mg/ kg + Aluminum chloride100 mg/kg	$8,83±2,64^{\gamma}$	$10,33±0,82^{\gamma}$	$1,33±0,82^{\alpha}$	$5,83±0,75^{\beta}$

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, aP<0.05, cP<0.001, significantly different compared to distilled water-treated group. γp<0.001, βp<0.01significant difference compared to aluminum chloride-treated group. ED, distilled water; Ac100, 100 mg/kg *Allium cameleon* aqueous extracts, AlCl: aluminum chloride 100 mg.

3.2. Effects of *Allium cameleon* on aluminum chloride-induced alterations in sperm count, viability, morphology and motility

The effects of AlCl3 on sperm count, viability, morphology and motility are summarized in Table 3. The table shows significant variability between the different groups studied [F (5, 29) = 824.37; p<0.001]. The table shows that AlCl3 significantly reduces sperm count (p<0.001), ranging from 54.85±3.10 x106/mL in rats treated with distilled water to 13.29±1.33 x106/mL in those treated with AlCl3. Treatment of animals simultaneously with AlCl3 and plant extract at a dose of 200 mg/kg reduces this number to 32.49±1.27 x106/mL. The plant preparation significantly increased sperm count (p<0.0001) to 61.08±1.15x106/mL. Similarly, a significant decrease (p< 0.0001) in sperm viability was observed

in animals treated with AlCl3 alone. Sperm viability was reduced from 77.02±2.07% in rats treated with distilled water to 29.63±1.35% ($p<0.001$) in those treated with AlCl3 at a dose of 100 mg/kg. Co-administration of AlCl3 and plant extract (200 mg/kg) increased this percentage to 63.19±3.49. Plant extract alone significantly increased the percentage to 91.47±3.05%. Similarly, AlCl3 reduced the percentage of sperm with normal morphology. This percentage ranged from 69.21±1.63% for the group treated with distilled water to 25.84±3.10% for animals given AlCl3 at a dose of 100 mg/kg. Simultaneous administration of *Allium cameleon* extract and AlCl3 increased this percentage to 57.49±3.65%. The plant itself increased this percentage to 82.67±2.06%. A decrease in sperm motility was also observed in animals treated with AlCl3. The percentage of sperm with normal motility, which was 73.24±1.01% in animals treated with distilled water, fell to 25.84±3.10% in those treated with AlCl3. Treatment of the animals with *Allium cameleon* leaf powder at a dose of 200 mg/kg increased the percentage of mobility to 88.47±1.84%. Captivatingly, sildenafil citrate had a positive effect on the parameters studied, both in co-administration with aluminum chloride and alone (Table 3).

Table 3 Effect of *Allium cameleon* on aluminum chloride-induced alterations in sperm count, viability, morphology and motility of male rats after daily treatment for 30 days.

Treatments	**sperm characteristics**			
	Count (10^6/mL)	**Mobility (%)**	**morphology (%)**	**Viability (%)**
Distilled water ml/kg	54,85±3,10$^{\gamma}$	73,24±1,01$^{\gamma}$	69,21±1,63$^{\gamma}$	77,02±2,07$^{\gamma}$
Aluminum chloride 100 mg/kg	13,29±1,33^{c}	30,31±3,66^{c}	25,84±3,10^{c}	29,63±1,35^{c}
Allium cameleon 200 mg/kg	61,08±1,15$^{c\gamma}$	88,47±1,84$^{c\gamma}$	82,67±2,06$^{c\gamma}$	91,47±3,05$^{c\gamma}$
Aluminum chloride 100 mg/kg + *Allium cameleon* 200 mg/kg	32,49±1,27$^{c\gamma}$	50,03±1,80$^{c\gamma}$	57,49±3,65$^{c\gamma}$	63,19±3,49$^{c\gamma}$
Sildenafil 60 mg/ kg	56,46±0,72$^{a\gamma}$	76,50±1,45$^{b\gamma}$	73,49±1,13$^{a\gamma}$	80,43±1,62$^{b\gamma}$
Sildenafil 60 mg/ kg + Aluminum chloride100 mg/kg	30,04±0,39$^{c\gamma}$	42,99±0,86$^{c\gamma}$	50,78±1,09$^{c\gamma}$	47,54±1,27$^{c\gamma}$

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, aP<0.05, bP<0.01, cP<0.001, significantly different compared to distilled water-treated group. γp<0.001, significant difference compared to aluminum chloride-treated group. ED, distilled water; Ac100, 100 mg/kg *Allium cameleon* aqueous extracts, AlCl: aluminum chloride 100 mg.

3.3. Effects of *Allium cameleon* on aluminum chloride-induced alterations in testicular, seminal vesicle and prostate weights

Table 4 summarizes the effects of AlCl3 on testicular, seminal vesicle and prostate weights. The table shows significant variability in testicular weight in the different groups studied [$F(5, 29) = 90.21$; $p<0.001$]. This table shows that AlCl3 significantly reduces testicular weight ($p<0.001$). Testicular weight fell from 1.67±0.05 g in the control lot treated with distilled water to 0.96±0.04 in the lot treated with AlCl3. In animals treated simultaneously with 200 mg/kg *Allium cameleon* and 100 mg/kg $AlCl_3$, this weight rose to 1.43±0.09 g ($p<0.001$). For the group treated with 200 mg/kg *Allium cameleon*, testicular weight increased to 2.07±0.08. Similarly, a significant decrease ($p<0.0001$) in seminal vesicle weight was observed in animals treated with AlCl3. This decreased from 0.99±0.01 g in rats treated with distilled water to 0.69±0.01 g in those treated with AlCl3. In animals treated concomitantly with plant extract and AlCl3, this weight increased significantly ($p<0.001$) compared to the AlCl3-treated group. Plant alone at 200 mg/kg increased this weight to 1.54±0.05 ($p<0.001$). Also, treatment with AlCl3 reduced ($p<0.001$) prostate weight from 0.57±0.02 for the control group treated with distilled water to 0.48±0.02 in animals given AlCl3. For animals treated simultaneously with aluminum chloride and 200 mg/kg of *Allium cameleon* leaf powder, prostate mass increased significantly ($p<0.001$) to 0.67±0.02 g. For animals treated with plant extract alone, this mass increased significantly to 0.90±0.06 g. The analyses show significant differences between the groups treated with sildenafil citrate and those treated only with AlCl3 (Table 4).

Table 4 Effect of Allium cameleon on aluminum chloride-induced alterations in testicular, seminal vesicle and prostate weights of male rats after daily treatment for 30 days.

Treatments	Sexual organs weight (g)		
	Testes	**Testes**	**Testes**
Distilled water ml/kg	1,67±0,05γ	0,99±0,01	0,57±0,02γ
Aluminum chloride 100 mg/kg	0,96±0,04c	0,69±0,01	0,48±0,02c
Allium cameleon 200 mg/kg	2,07±0,08cγ	1,54±0,05cγ	0,90±0,06cγ
Aluminum chloride 100 mg/kg + *Allium cameleon* 200 mg/kg	1,43±0,09aγ	1,05±0,04cγ	0,67±0,02γ
Sildenafil 60 mg/ kg	2,23±0,24cγ	1,45±0,19cγ	0,91±0,06cγ
Sildenafil 60 mg/ kg + Aluminum chloride100 mg/kg	1,37±0,11bγ	0,98±0,13c	0,64±0,004γ

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, aP<0.05, bP<0.01, cP<0.001, significantly different compared to distilled water-treated group. γp<0.001, significant difference compared to aluminum chloride-treated group. ED, distilled water; Ac100, 100 mg/kg *Allium cameleon* aqueous extracts, AlCl: aluminum chloride 100 mg.

3.4. Effects of *Allium cameleon* on aluminum chloride-induced alterations in plasma testosterone, FSH and LH levels

Table 5 summarizes the effects of AlCl3 on plasma testosterone, FSH and LH levels. The table shows significant variability in testosterone levels between treatments [F (5, 30) = 110.69; p<0.001]. The post hoc test showed a significant decrease (p<0.001) in testosterone levels, ranging from 10.04±0.91 for the control treated with distilled water to 5.47±0.91 for the animals treated with AlCl3. Simultaneous treatment of animals with AlCl3 and plant extract significantly increased this level to 10.15±0.54. *Allium cameleon* administered alone at a dose of 200 mg/kg significantly increased the rate to 14.96±1.50. The table also shows a significant variation in FSH levels between treatments [F (5, 30) = 62.22; p < 0.001]. The post hoc test shows a significant decrease (p<0.001) in FSH levels, from 12.75±1.54 for the control treated with distilled water to 4.14±1.59 for the animals treated with AlCl3. Simultaneous treatment of animals with AlCl3 and plant extract significantly increased this rate to 10.97±1.17. *Allium cameleon* administered alone at 200 mg/kg significantly increased the rate to 15.86±1.61. Similarly, significant variability in LH levels was observed between the different groups [F (5, 30) = 66.47; p<0.001]. The table shows that LH levels were significantly lower (p<0.001) in animals treated with AlCl3 than in those treated with distilled water. This level, which was 13.83±2.36 in rats treated with distilled water, fell to 3.96±1.60 in those treated with AlCl3. In animals treated simultaneously with AlCl3 and plant extract, an increase (p<0.001) in LH levels was observed compared with animals treated with AlCl3 alone. This increased from 3.96±1.60 in rats treated with AlCl3 to 16.89±1.06 in those treated with 200 mg/kg of the plant. The values 13.76±0.94, 14.07±0.94 and 15.16±0.85 obtained respectively for testosterone, FSH and LH with Sildenafil citrate (60 mg/kg) are all below the values of the 200 mg/kg dose of the plant. Also, co-administration of sildenafil and AlCl3 significantly increased the levels of these hormones compared with animals treated with AlCl3 alone (Table 5).

Table 5 Effect of *Allium cameleon* on aluminum chloride-induced alterations in plasma testosterone, FSH and LH levels of male rats after daily treatment for 30 days.

Treatments	Hormone		
	Testosterone (ng/ml)	**FSH (ng/ml)**	**LH (ng/ml)**
Distilled water ml/kg	10,04±0,91γ	12,75±1,54γ	13,83±2,36γ
Aluminum chloride 100 mg/kg	5,47±0,91c	4,14±1,59c	3,96±1,60c
Allium cameleon 200 mg/kg	14,96±1,50cγ	15,86±1,61bγ	16,89±1,06bγ
Aluminum chloride 100 mg/kg + *Allium cameleon* 200 mg/kg	10,15±0,54γ	10,97±1,17γ	15,02±1,36γ
Sildenafil 60 mg/ kg	13,76±0,94cγ	14,07±0,94γ	15,16±0,85γ
Sildenafil 60 mg/ kg + Aluminum chloride100 mg/kg	9,34±0,64γ	9,56±0,28bγ	9,51±1,04

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, aP<0.05, bP<0.01, cP<0.001, significantly different compared to distilled water-treated group. γp<0.001, significant difference compared to aluminum chloride-treated group. ED, distilled water; Ac100, 100 mg/kg *Allium cameleon* aqueous extracts, AlCl: aluminum chloride 100 mg.

3.5. Effect of *Allium cameleon* on MDA and GSH levels, SOD and catalase activities alteration induce by Aluminum chloride of male rats after daily treatment for 30 days

Table 6 summarizes the effects of AlCl3 on MDA and GHS levels. The analysis reveals significant variability in malondialdehyde levels [F (5, 30) = 39.38; p<0.001]. MDA levels increased significantly (p<0.001) from 1.70±0.02 in the distilled water-treated control to 1.42±0.21 in the AlCl3-treated animals. This rate of 1.71±0.25 was significantly (p<0.001) lower in animals treated simultaneously with plant extract and AlCl3 than in those treated with AlCl3 alone. Compared with this same group, the plant reduced (p<0.001) this rate to 1.12±0.06 in animals treated with 200 mg/kg. The table also reveals a significant reduction in reduced glutathione levels. GSH levels fell (p<0.001) from 2.37±0.16 in animals treated with distilled water to 1.31±0.22 in those treated with AlCl3. Compared to the AlCl3-treated control group, in animals treated simultaneously with plant extract and AlCl3, GSH levels increased significantly (p<0.001) to 2.30±0.15. For the group treated with plant extract alone, GSH levels were 3.03±0.15, and this increase was significantly different from that of the group treated with AlCl3. Analysis of this table also shows that sildenafil citrate, both alone and co-administered with AlCl3, significantly (p<0.001) reduced MDA levels to 1.37±0.07 and 1.85±0.16 respectively. Also, with these same treatments, a significant increase (p<0.001) in GSH levels was observed, to 3.05±0.51 and 2.11±0.11 respectively. This Table 6 summarizes also the effects of AlCl3 on catalase and superoxide dismutase activity. The analysis reveals significant variability in superoxide dismutase activity [F (5, 30) = 101.41; p<0.001]. SOD activity decreased significantly (p<0.001) from 1.14±0.06 in the control group treated with distilled water to 0.46±0.18 in the animals treated with AlCl3. This activity, which was 2.71±0.14, increased significantly (p<0.001) in animals treated simultaneously with plant extract and AlCl3, compared with those treated with AlCl3 alone. Compared to this same group, *Allium cameleon* increased (p<0.001) this activity to 3.79±0.58 in animals treated with 200 mg/kg. This table also reveals a significant decrease in catalase activity. In fact, catalase activity decreased (p<0.001) from 88.68±2.59 for animals treated with distilled water to 52.63±6.96 for those treated with AlCl3. Compared with the control group treated with AlCl3, in animals treated simultaneously with plant extract and AlCl3, catalase activity increased significantly (p<0.001) to 83.04±4.24. For the group treated with plant extract alone, catalase activity was 113.47±5.46, and this increase was significantly different from that of the group treated with AlCl3. Analysis of this table also shows that sildenafil citrate, both alone and co-administered with AlCl3, significantly (p<0.001) increased superoxide dismutase activity by 3.01±0.10 and 2.50±0.38 respectively. Also, with these same treatments a significant (p<0.001) increase in catalase activity was observed, to 102.73±11.24 and 80.72±1.95 respectively.

Table 6 Effect of *Allium cameleon* on MDA and GSH levels, SOD and catalase activities alteration induce by Aluminum chloride of male rats after daily treatment for 30 days

Treatments	***In vivo* Oxidative stress parameters**			
	MDA (mol/g)	SOD (U/g)	MDA (mol/g)	GSH (mol/g)
Distilled water ml/kg	1,14±0,06$^{\gamma}$	88,68±2,59$^{\gamma}$	1,70±0,02$^{\gamma}$	2,37±0,16$^{\gamma}$
Aluminum chloride 100 mg/kg	0,46±0,18^{c}	52,63±6,96^{c}	2,07±0,08^{c}	1,31±0,22^{c}
Allium cameleon 200 mg/kg	3,79±0,58$^{c\gamma}$	113,47±5,46$^{c\gamma}$	1,12±0,06$^{c\gamma}$	3,03±0,15$^{c\gamma}$
Aluminum chloride 100 mg/kg + *Allium cameleon* 200 mg/kg	2,71±0,14$^{c\gamma}$	83,04±4,24$^{\gamma}$	1,71±0,25$^{\gamma}$	2,30±0,15$^{\gamma}$
Sildenafil 60 mg/ kg	3,01±0,10$^{c\gamma}$	102,73±11,24$^{b\gamma}$	1,37±0,07$^{b\gamma}$	3,05±0,51$^{c\gamma}$
Sildenafil 60 mg/ kg + Aluminum chloride100 mg/kg	2,50±0,38$^{c\gamma}$	80,72±1,95$^{\gamma}$	1,85±0,16	2,11±0,11$^{\gamma}$

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, aP<0.05, bP<0.01, cP<0.001, significantly different compared to distilled water-treated group. γp<0.001, significant difference compared to aluminum chloride-treated group. ED, distilled water; Ac100, 100 mg/kg *Allium cameleon* aqueous extracts, AlCl: aluminum chloride 100 mg.

3.6. Effect of *Allium cameleon* on penile Nitric oxide and cGMP levels of male rats after daily treatment for 30 days

Figure 2 shows the effects of AlCl3 on penile nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) levels. The analysis revealed significant variability in cGMP levels [F (5, 30) = 60.88; p<0.001]. The level of cGMP decreased significantly (p<0.001) from 12.65±1.06 in the control treated with distilled water to 6.14±1.26 in the animals treated with AlCl3. This rate of 11.01±0.76 was significantly (p<0.001) higher in animals treated simultaneously with *Allium cameleon* extract and AlCl3 than in those treated with AlCl3 alone. Compared to this same group, the plant increased (p<0.001) this rate to 14.25±1.38 in animals treated with 200 mg/kg. The table also reveals a significant decrease in NO levels. In fact, NO levels decreased (p<0.001) from 10.63±0.68 for animals treated with distilled water to 4.86±0.50 for those treated with AlCl3. Compared with the AlCl3-treated control group, in animals treated simultaneously with plant extract and AlCl3, NO levels rose significantly (p<0.001) to 10.18±0.49. For the group treated with plant extract alone, the NO level was 15.54±1.57, and this increase was significantly different from that of the group treated with AlCl3. Analysis of this table also shows that sildenafil citrate, both alone and co-administered with aluminum chloride, significantly (p<0.001) increased cGMP levels to 14.81±1.55 and 10.11±0.85 respectively. The same treatments also significantly (p<0.001) increased NO levels, to 15.01±0.93 and 9.33±0.45 respectively.

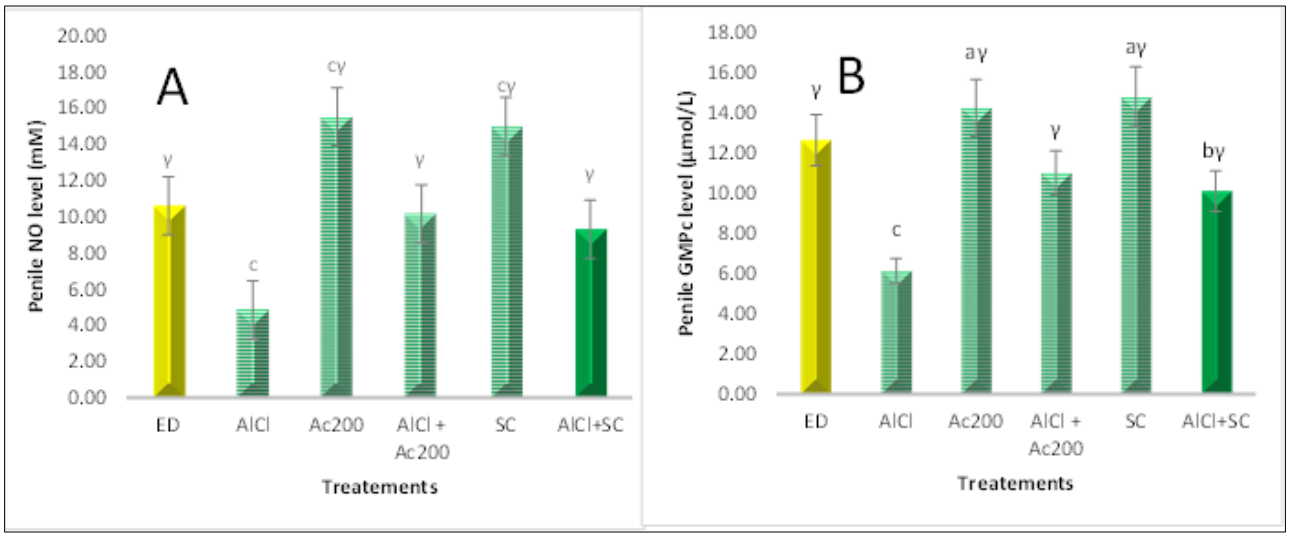


Figure 2 Effects of *Allium cameleon* extracts on altered penile level of nitric oxide (A) and cGMP concentration (B) of male rats induced by aluminum chloride after daily treatment for 30 days

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, aP<0.05, bP<0.01, cP<0.001, significantly different compared to distilled water-treated group. γp<0.001, significant difference compared to aluminum chloride-treated group. ED, distilled water; Ac100, 100 mg/kg *Allium cameleon* aqueous extracts, AlCl: aluminum chloride 100 mg.

3.7. Effects of *Allium cameleon* on aluminum chloride-induced alterations in serum levels of tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6) and interleukin 1 beta (IL1-β)

Table 7 summarizes the effects of AlCl3 on TNFα, IL-6 and IL1-β levels. The analysis reveals significant variability in TNFα levels [F (5, 30) = 50866.78; p<0.001]. TNFα levels increased significantly (p<0.001) from 235.31±0.34 in the distilled water-treated control to 332.43±0.25 in the AlCl3-treated animals. This rate of 156.25±1.77 was significantly (p<0.001) lower in animals treated simultaneously with plant extract and AlCl3 than in those treated with AlCl3 alone. Compared with this same group, the plant reduced (p<0.001) this rate to 107.94±1.01 in animals treated with 200 mg/kg. The table also reveals a significant increase in IL-6 levels. IL-6 levels increased (p<0.001) from 338.83±2.06 in animals treated with distilled water to 476.60±1.20 in those treated with AlCl3. Compared with the AlCl3-treated control group, in animals treated simultaneously with plant extract and AlCl3, IL-6 levels were significantly reduced (p<0.001) to 156.25±1.77. For the group treated with plant extract alone, IL-6 levels were 146.94±1.20, and this decrease was significantly different from that of the group treated with AlCl3. This table also reveals a significant increase in the level of IL1-β levels. Indeed, IL1-β levels increased (p<0.001) from 183.20±3.14 in animals treated with distilled water to 464.72±3.59 in those treated with AlCl3. Compared with the AlCl3-treated control group, in animals treated simultaneously with plant extract and AlCl3, IL1-β levels were significantly reduced (p<0.001) to 140.94±1.10. The IL1-β level in the group treated with plant extract alone was 122.60±1.25, and this decrease was significantly

different from that in the group treated with AlCl3. Analysis of this table also shows that sildenafil citrate, both alone and co-administered with AlCl3, significantly ($p<0.001$) reduced pro-inflammatory cytokine levels.

Table 7 Effect of *Allium cameleon* on serum levels of tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6) and interleukin 1 beta (IL1-β) induced by aluminum chloride in male rats after daily treatment for 30 days

Treatments	**Cytokines**		
	TNFα (pg/mL)	**IL-6 (pg/mL)**	**IL-1β (pg/mL)**
Distilled water ml/kg	235,31±0,34$^{\gamma}$	338,83±2,06$^{\gamma}$	183,20±3,14$^{\gamma}$
Aluminum chloride 100 mg/kg	332,43±0,25^{c}	476,60±1,20^{c}	464,72±3,59^{c}
Allium cameleon 200 mg/kg	107,94±1,01$^{c\gamma}$	146,94±1,20$^{c\gamma}$	122,60±1,25$^{c\gamma}$
Aluminum chloride 100 mg/kg + *Allium cameleon* 200 mg/kg	156,25±1,77$^{c\gamma}$	125,75±0,87$^{c\gamma}$	140,94±1,10$^{c\gamma}$
Sildenafil 60 mg/ kg	200,84±3,62$^{c\gamma}$	97,84±0,83$^{c\gamma}$	130,88±1,09$^{c\gamma}$
Sildenafil 60 mg/ kg + Aluminum chloride100 mg/kg	244,86±0,96$^{c\gamma}$	137,86±2,05$^{c\gamma}$	156,03±1,13$^{c\gamma}$

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, $aP<0.05$, $bP<0.01$, $cP<0.001$, significantly different compared to distilled water-treated group. $\gamma p<0.001$, significant difference compared to aluminum chloride-treated group. ED, distilled water; Ac100, 100 mg/kg *Allium cameleon* aqueous extracts, AlCl: aluminum chloride 100 mg.

4. Discussion

The fact that certain heavy metals such as aluminum are associated with sexual dysfunction has been established by several studies [23]. Aluminum is only present in trace amounts in biological systems [10]. It has been shown to be poorly absorbed and efficiently eliminated when absorption occurs; it is mainly described in bone, liver, testis, kidney and brain. Its accumulation in tissues and organs leads to dysfunction and toxicity [23]. Some of the pathological manifestations of sexual dysfunction in men include altered sexual behavior, impaired erectile function, oxidative damage to endothelial cells and inflammatory reactions [10], [13], [24]. Toxicity on the male reproductive system occurs due to the accumulation of Aluminum and are due to by various mechanisms such as induction of oxidative stress, interference with spermatogenesis and steroidogenesis, alteration of cell signaling, disruption of the blood-testicular barrier, and affect the endocrine system [13]. Since time immemorial, plants have been used worldwide by various cultures as a safe and effective natural source of drugs for the treatment of male infertility [25],[26]. The use of an animal model for screening to determine the potentials of a drug substance is a recognized model. Moreover, using rats to mimic and understand the possible effects of consumed elements is simple and rapid [8]. The present study shows the toxicity of aluminum chloride on sexual behavior; on sperm count, morphology, viability and motility; on the weight of certain sexual organs; and on the concentration of testosterone, FSH and LH. It also demonstrates the efficacy of *Allium cameleon* in reducing the deleterious effects of aluminum.

In the present investigation, administration of aluminum chloride resulted in an alteration of the animals' sexual behavior, such as ejaculation latency, frequency of mounting, intromission, ejaculation and erection, indicating a decreased desire for copulation in male rats. An increase in mounting latency, intromission latency and post-ejaculatory interval was also observed, indicating a decrease in sexual arousal in male rats. These results reflect a deterioration in performance, motivation, vigor and degree of sexual stimulation, corroborating the findings of previous authors [27], [28], confirming deterioration in the sexual function of male rats. However, treatment of these rats with *Allium cameleon* extract reversed these effects and restored sexual activity in these animals, suggesting antagonism of the toxic effects of aluminum by compounds contained in the plant extract. These deleterious effects could be attributed to a drop in testosterone levels, which are responsible for sexual behavior. The relationship between Testosterone synthesis and sexual behavior in male mice has been proven in a previous study [29]. The present study also shows that aluminum chloride induces a reduction in sperm count, viable sperm rate, normal morphology and motility as well as a decrease in testicular, seminal vesicle and prostate weights. These results corroborate those of other authors [30], [31], [32] who showed that aluminum chloride-treated animals had significantly lower testicular, seminal vesicle and prostate weights than control animals. This decrease in organ weight may be due to mitochondrial dysfunction and disruption of glucose metabolism, as mitochondria would be one of the possible targets of aluminum's harmful effects [33]. Aluminum accumulation in the testes can have deleterious effects on these reproductive organs through degeneration and cell

death, reduction in tubule size, arrest of spermatogenesis and inhibition of steroid hormone biosynthesis by Leydig cells resulting in atrophy of the testes, epididymis and sex glands [34], [35]. These effects were reversed by treatment with *Allium cameleon* leaf extract.

The plant extract may stimulate tissue regeneration and therefore have a positive impact on the weight of these organs. Knowing that the increase in the weight of sexual organs is probably a consequence of an increase in the functional activity of these organs leading to an increase in their secretory capacity [36], and that these organs are androgen-dependent organs, plant extract may also increase the availability of androgens, thus promoting their normal functioning. Our results indicate a significant decrease in sperm count, motility and viability in the aluminum chloride-treated group. Metals have been shown to affect spermatogenesis, which can lead to low sperm count, abnormal morphology, low viability and reduced sperm motility [10]. Rats treated with *Allium cameleon* leaf extract showed higher sperm count, motility, normal morphology and viability. Motility is one of the most important characteristics of fertile spermatozoa, widely used as an indicator of sperm function. Motility is one of the most important characteristics of fertile spermatozoa, widely used as an indicator of sperm function. Sperm motility is an important attribute, as it is easily identifiable and reflects several structural and functional competencies, as well as key aspects of sperm metabolism [37]. The integrity of the plasma membrane is demonstrated by the ability of a viable cell to exclude the dye, whereas the dye diffuses passively into spermatozoa with a damaged plasma membrane [37]. Thus, the plant extract could restore and stimulate the aluminum chloride-altered spermatogenesis process in the testis, and improve sperm quality in the extract-treated group. These results suggest that *Allium cameleon* extract may have pro-fertility effects.

The results of this work show that aluminum chloride affects not only exocrine function, but also endocrine function. Testosterone, FSH and LH levels were significantly reduced in animals treated with aluminum chloride. These results are in line with those of other authors who have observed a decrease in the concentration of sex hormones in rats treated with aluminum chloride [24], [30], [31], [38]. An increase in testosterone, FSH and LH concentration was observed in animals treated with *Allium cameleon* extract compared to animals treated with aluminum chloride. This suggests that the plant extract may be involved in the synthesis of these hormones. These observations could be the result of aluminum chloride's pro-oxidant and pro-inflammatory activities, with the plant acting as an antioxidant and anti-inflammatory.

In parallel with previous studies, aluminum chloride administration induced an increase in MDA pro-inflammatory cytokines and a reduction in GSH levels and superoxide dismutase and catalase enzyme activities. Indeed, it is known that aluminum can act as a pro-oxidant and thereby disrupt enzymatic activities [39] through the elevation of ROS levels leading to cellular senescence, a mechanism that occurs after cell proliferation arrest in response to cellular damage occurring during replication [40]. Moreover, aluminum circulates in the blood mainly bound to transferrin, so it has the ability to disrupt iron homeostasis by displacing it from transferrin, resulting in iron release into the blood and the generation of free radicals [41], [42]. Indeed, it has been reported that aluminum can induce generalized inflammation in the host and affect Leydig cells causing a drop in serum testosterone levels, which alters the integrity of the blood-testis barrier [43]. Also, this can induce histological alterations such as vacuolization and degenerative changes in seminiferous tubules [44], [43], [45]. Previous studies have shown an increase in tumor necrosis factor-α (TNF-α), interliukin-1α (IL-1α) and interliukin-6 (IL-6) in aluminum-treated male rats [30]. Park et al., [46] demonstrated that aluminum nanoparticles induced a significant increase in IL-6 inflammatory cytokines. In addition, Rodrigo et al., [47] reported that aluminum nanoparticles have been shown to initiate inflammatory events, including the secretion of pro-inflammatory cytokines. However, *Allium cameleon* induced a decrease in lipid peroxidation and pro-inflammatory cytokines, as well as an increase in GSH levels and the enzymatic activity of superoxide dismutase and catalase. All these observations could be the consequence of *Allium cameleon*'s anti-inflammatory, antioxidant and/or antioxidant enzyme expression effects, or that certain substances contained in the plant act directly as pro-fertilizers.

5. Conclusion

It appears from this research that adverse effects of aluminum chloride on testicular function, resulting in altered sexual behavior, sperm parameters, sex organ weight, serum testosterone, FSH and LH levels. These effects are thought to be caused by altered gene expression of proteins involved in mitochondrial biogenesis and function, induction of oxidative stress, lipid peroxidation, apoptotic and necrotic cell death pathways in testicular tissue. Similarly, induction of the inflammatory pathway through increased production of tumor necrosis factor α, interleukin-1β and interleukin-6 and imbalance of sex hormones. Furthermore, the present study showed that *Allium cameleon* extract effectively antagonizes the damaging effects of aluminum chloride. These protective effects may be the result of antioxidant and anti-inflammatory effects, or the presence in the plant extract of particles which directly mimic the effects of certain endogenous molecules.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

The protocols were performed in concordance with the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee, Yaoundé (No. FW-IRB00001954).

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