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Allium cameleon extract improved androgenesis and sexual behavior in male rat (*Rattus norvegicus*) via attenuation of oxidative damages increased sexual hormone and NO/cGMP cascade

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Abstract

Objective: *Allium cameleon* Cronquist (1981) (Liliaceae) is a bulb belonging to the garlic family, known as a medicinal plant used for its aphrodisiac activities in Cameroonian folk medicine. The present study was conducted to assess the aphrodisiac potentials of *Allium cameleon* aqueous extracts and investigate on the possible mechanisms by which this could be accomplished.

Methods: The behavioral and biochemical tests were used to detect the aphrodisiac properties. The conceivable mechanisms of action of the aqueous extracts were investigated through determination of sexual hormones, nitric oxide, cGMP, and antioxidant activities in male rats treated with different plant extract concentrations.

Results: *Allium cameleon* enhanced sexual excitement, improved semen quality, and the concentrations of testosterone, FSH and LH. Levels of endogenous antioxidant enzymes (CAT, SOD, and GSH) were improved. 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.

Conclusion: These findings suggest that *Allium cameleon* possess aphrodisiac properties in rats that might be involve in the attenuation of oxidative damage, but also in the increase of sexual hormones production and NO/cGMP cascade.

Keywords: *Allium cameleon*; Aphrodisiac; Oxidative damage; Sexual hormones; Folk medicine

1. Introduction

Infertility is defined by the WHO as the absence of pregnancy despite regular unprotected sexual activities for more than one year without contraception [1]. The infertility of male has for a long time been underrecognized by scientist and clinicians, although over time, the scientific community is raising consciousness of male infertility as a serious medical and public health problem [2]. In approximately a half of all couples who are affected by infertility, the male partner has a deficiency in his sperm [3]. The causes of male infertility are numerous and wide in range, and include obstructive, hormonal, immunological and varicocele problems. However, in more than 25% of cases, no identified cause is found, in the called idiopathic infertility, in which the probable causes involve the oxidative stress [4]. Indeed,

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morphologically abnormal spermatozoa have an increased capacity to generate reactive oxygen species, but also have a much-reduced antioxidant capacity [5]. Infertility has a strong social impact such as divorce, a highly negative impact on self-esteem, increasing risk of depression, distress, anxiety, somatic complaints, reduced libido, as well as a sense of blame and guilt [6-8]. As a sickness, many treatment efforts have been suggested. Modern treatments include several drugs such as sildenafil citrate, clomiphene citrate and anastrozole. These drugs have significant side effects including marked anxiety, sleep disturbances, headaches, visual disturbances, dizziness, hot flashes, mood swings, irritability, emotionality, nausea, feeling tired, fainting, stroke, heart attack, ... [9]. In recent years, complementary and alternative medicine has regained interest in the treatment of various diseases, including male infertility [10]. The mechanisms involved in the beneficial effects of medicinal plants in the treatment of infertility include antioxidant, anti-inflammatory, anti-edematous and venotonic activities, as well as the presence of precursors for sperm production and the increased blood testosterone levels. This study was investigated to assess the efficacy of *Allium cameleon* extract in treating sperm abnormalities, attenuating oxidative damages through increased sexual hormone and NO/cGMP cascade.

2. Material and methods

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's 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 obtain two less concentrated solutions of 15 and 10 mg/mL.

2.3. Assessment of the sexual behavior of male rats

Sixty rats (thirty sexually active male (220–250 g) and thirty 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 *ad libitum*. Rats were randomly divided into five groups. Each group contained 6 rats. Group I and 5 served as control and received distilled water and sildenafil citrate 60 mg/kg respectively. Group 2, group 3 and group 4 received 100 mg/kg, 150 mg/kg and 200 mg/kg body weight of *Allium cameleon* aqueous extract respectively. 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*. [11]. 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 [11].

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*. [12]. To assess the motility, 20 µl was used following the scale basis as described by Mohammed and Engidawork [13]. Sperm viability expressed as percentage of swollen semen was analyzed using hypo-osmotic swelling test [14], 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 [15]. 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 [16]. 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 [17]. 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 [18].

2.7.4. Glutathione (GSH) Assay

The method described by Beutler *et al.* [19] 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. 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. Effect of *Allium cameleon* on the behavior of male rats

3.1.1. Effect of Allium cameleon on mount, intromission and ejaculation latencies, and post ejaculatory interval

Table 1 Effect of *Allium cameleon* extract on mount, intromission and ejaculatory latencies, and post ejaculatory interval of male rats after daily treatment for 30 days

Treatments	Sexual behavior parameters (s)			Post ejaculatory interval
	Mount Latency	Intromission Latency	Ejaculation Latency	
Distilled water 10 ml/kg	98.17±3.76	111.33±2.80	212.33±1.51	422.50±4.42
Allium cameleon 100 mg/kg	87.67±4.32[c]	86.50±1.87[c]	262.00±2.61[c]	347.50±2.43[c]
Allium cameleon 150 mg/kg	76.33±1.75[c]	65.50±1.64[c]	276.17±1.94[c]	298.17±7.63[c]
Allium cameleon 200 mg/kg	47.50±3.62[c]	55.67±1.03[c]	284.17±1.72[c]	265.17±1.72[c]
Sildenafil 60 mg/ kg	43.17±1.47[c]	55.33±1.21[c]	290.00±4.29[c]	251.00±1.67[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, significantly different compared to the negative control group.

The administration of *Allium cameleon* aqueous extracts significantly decrease mount [F (5, 28) = 122.11, p<0.001] and intromission [F (5, 28) = 122.11, p<0.001] latencies of rats when compared with the control treated with distilled water. Mounts latency varied from 98.17±3.76 for distilled water treated group to 76.33±1.75 and 47.50±3.62 for 150 mg/kg and 200 mg/kg plant treated group respectively (Table 1). Interestingly, *Allium cameleon* extracts administered at all doses and Sildenafil citrate (60 mg/kg) as reference, significantly increased ejaculation latency [F (5, 28) = 122.11, p<0.001] and post ejaculatory interval [F (5, 28) = 122.11, p<0.001] of rats when compared with the control treated with distilled water (Table 1).

3.1.2. Effect of Allium cameleon on mount, intromission, ejaculatory and erection frequencies.

Table 2 shows the effects of *Allium cameleon* on mount, intromission, ejaculatory and erection latencies of male rats after 30 days treatments. It appears from this table, an increase of mount frequency varying from 11.83±1.47 for distilled water treated group to 19.83±1.83 and 22.83±1.17 for the group treated by 200 mg/kg and sildenafil citrate 60 mg/kg was respectively observed. Post hoc analysis indicated a significant difference between treatments [F (5, 28) = 149.34, p<0.001]. In the similar manner, intromission frequency expands from 10.17±0.75 for negative control to 15.67±1.75 for *Allium cameleon* at dose 200 mg/kg, and 16.00±1.26 for the positive control treated with sildenafil citrate at 60 mg/kg. Statistical analysis displayed a significant difference [F (5, 28) = 87.90, p<0.001] as compared to negative control. Interestingly, ejaculatory frequency also increased from 1.33±0.52 for negative control to 2.83±0.75 and 2.67±0.52 respectively for the group treated at dose 200 mg/kg of the plant extract and with sildenafil citrate treated group. Data analysis reveal a significant difference [F (5, 28) = 7.72, p<0.001] as compared to distilled water treated group. Moreover, the erection frequency was enhanced by both *Allium cameleon* extract and sildenafil citrate varying from 6.67±0.82 for distilled water treated group to 14.50±1.05 and 15.67±1.21 respectively for the group treated the dose 200 mg/kg of the plant extract and sildenafil citrate at 60 mg/kg. Post hoc analysis disclosed significant variation [F (5, 28) = 7.72, p<0.001] as compared to negative control treated with distilled water.

Table 2 Effect of *Allium cameleon* extract on mount, intromission, ejaculatory and erection frequencies of male rats after daily treatment for 30 days

Treatments	Sexual behavior frequency			Erection
	Mount	Intromission	Ejaculation	
Distilled water 10 ml/kg	11.83±1.47	10.17±0.75	1.33±0.52	6.67±0.82
Allium cameleon 100 mg/kg	13.17±0.75[a]	12.50±1.38[a]	2.33±0.52[c]	9.00±0.89[a]
Allium cameleon 150 mg/kg	15.67±1.21[c]	12.83±0.75[b]	2.67±0.52[c]	12.67±1.21[c]
Allium cameleon 200 mg/kg	19.83±1.83[c]	15.67±1.75[c]	2.83±0.75[c]	14.50±1.05[c]
Sildenafil 60 mg/kg	22.83±1.17[c]	16.00±1.26[c]	2.67±0.52[c]	15.67±1.21[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, [a]P<0.05, [b]P<0.01, [c]P<0.001, significantly different compared to the negative control group.

3.2. Effect of *Allium cameleon* on Sexual organs weight, sperm count, motility, morphology and viability

3.2.1. Effect of Allium cameleon on Sexual organs weight of male rats after daily treatment for 30 days

Table 3 Effect of *Allium cameleon* extract on Sexual organs weight of male rats after daily treatment for 30 days

Treatments	Sexual organs weight (g)		
	Testes	Seminal vesicles	Prostate
Distilled water 10 ml/kg	1.67±0.05	0.99±0.01	0.57±0.02
Allium cameleon 100 mg/kg	1.96±0.03[a]	1.03±0.06	0.67±0.03[a]
Allium cameleon 150 mg/kg	2.20±0.19[c]	1.36±0.07[c]	0.73±0.04[c]
Allium cameleon 200 mg/kg	2.07±0.08[c]	1.54±0.05[c]	0.90±0.06[c]
Sildenafil 60 mg/kg	2.23±0.24[c]	1.45±0.19[c]	0.91±0.06[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, [a]P<0.05, [c]P<0.001, significantly different compared to the negative control group.

The aqueous extract produced an increase in testes weight ranging significantly from 1.67±0.05 g for the negative control to 2.20±0.1 and 2.23±0.24 g for the group treated with dose 150 mg/kg of the plant and sildenafil citrate respectively (Table 3). Post hoc study revealed a significant variation [F (4, 25) = 15.17, p<0.001] of testes weight as compared to negative control group. For seminal vesicles, a dose dependent increase of weight was observed ranging from 0.99±0.01 for the negative control to 1.54±0.05 and 1.45±0.19 g at dose 200 mg/kg of *Allium cameleon* and sildenafil citrate 60 mg/kg respectively. Analysis shows significant variation [F (4, 25) = 38.58, p<0.001] of seminal vesicles weight as compared to distilled water treated group. Similarly, the prostate weight follows a dose dependent pattern shifting from 0.57±0.02 g for the negative control to 0.90±0.06 and 0.91±0.06 g at dose 200 mg/kg treated group and sildenafil citrate respectively (Table 3). As compared to negative control, statistical analysis showed a significant variation of the whole [F (4, 25) = 38.58, p<0.001].

3.2.2. Effect of Allium cameleon on sperm count, motility, morphology and viability of male rats after daily treatment for 30 days

The effects of the extracts on sperm characteristics are summarized in Table 4. Daily treatment for 30 days followed by sperm count, mobility, morphology and viability indicated an increase in all aspects of sperm characteristics in the *Allium cameleon*-treated animals compared to negative controls group. An increase of sperms count in a dose dependent manner was observed. This number significantly varied [F (4, 25) = 11.94, p<0.001] from 54.85±3.10 x10^6/mL for the distilled water treated group to 61.08±1.15 and 56.46±0.72x x10^6/mL at dose 200 mg/kg of the plant and positive control respectively. The percentage of mobility was furthermore dose dependent, varying from 73.24±1.01 to 88.47±1.84 and 76.50±1.45 % respectively at dose 200 mg/kg of *Allium cameleon* and the positive control. The post hoc analysis revealed significant difference [F (4, 25) = 34.32, p<0.001] compared to distilled treated group. Equivalently, the morphology percentage with the plant extract doses, from 69.21±1.63 for the negative control to 82.67±2.06% at dose 200 mg/kg. The percentage of viability fluctuated from 77.02±2.07 to 91.47±3.05% respectively for distilled water and at dose 200 mg/kg of the plant treated groups. Post hoc analysis showed significant differences [F (4, 25) = 31.18, p<0.001] and [F (4, 25) = 27.08, p<0.001], corresponding respectively for morphology percentage and viability percentage probabilities elements as compared to negative control.

Table 4 Effect of *Allium cameleon* extract on sperm count, motility, morphology and viability of male rats after daily treatment for 30 days

Treatments	sperm characteristics			
	Count (10^6/mL)	Mobility (%)	Morphology (%)	Viability (%)
Distilled water 10 ml/kg	54.85±3.10	73.24±1.01	69.21±1.63	77.02±2.07
Allium cameleon 100 mg/kg	56.76±0.90[a]	73.96±3.10	71.62±2.60[a]	78.97±2.35[a]
Allium cameleon 150 mg/kg	57.20±1.06[b]	75.23±4.27[b]	74.23±3.16[b]	84.25±3.79[c]
Allium cameleon 200 mg/kg	61.08±1.15[c]	88.47±1.84[c]	82.67±2.06[c]	91.47±3.05[c]
Sildenafil 60 mg/kg	56.46±0.72[a]	76.50±1.45[b]	73.49±1.13[a]	80.43±1.62[b]

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, [a]P<0.05, [c]P<0.001, significantly different compared to the negative control group.

3.3. Effect of *Allium cameleon* on plasmatic testosterone, FSH and LH level of male rats after daily treatment for 30 days

The effects of *Allium cameleon* on plasmatic testosterone, FSH and LH level are presented in table 5. The increase level in blood testosterone varied from 10.04±0.91 for the negative control to 14.96±1.50 and 13.76±0.94 respectively for the dose 200 mg/kg of *Allium cameleon* and sildenafil citrate. Significant differences [F (4, 25) = 22.50, p < 0.001] were observed between treatments as compared to negative control. Administration of *Allium cameleon* increased plasmatic level of FSH varying from 12.75±1.54 for the distilled water treated group to 15.86±1.61 for the 200 mg/kg of the plant treated group. It appears from post hoc analysis a significant variation of FSH level in the whole [F (4, 25) = 4.54, p<0.01]. For the luteinizing hormone, a dose dependent increase was observed, the plasmatic level of this hormone varies from 13.83±2.36 ng/ml for distilled water treated group to 16.89±1.06 ng/ml for the group of animals treated at dose 200 mg/kg of *Allium cameleon*. Post hoc analysis displayed a significant variation [F (4, 25) = 4.54, p<0.01].

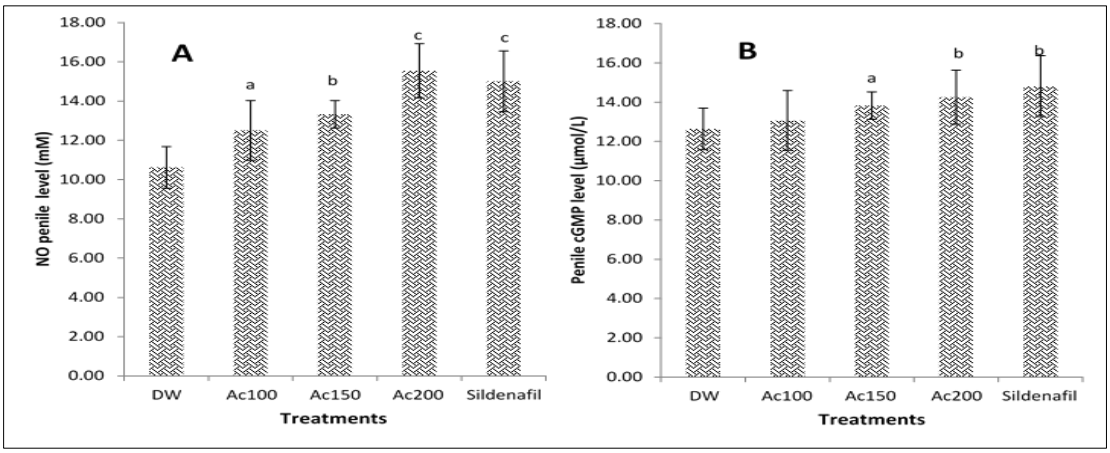
Table 5 Effect of *Allium cameleon* extract on serum testosterone, FSH and LH concentrations of male rats after daily treatment for 30 days

Treatments	Hormone		
	Testosterone (ng/ml)	FSH (ng/ml)	LH (ng/ml)
Distilled water 10 ml/kg	10.04±0.91	12.75±1.54	13.83±2.36
Allium cameleon 100 mg/kg	10.84±0.70	12.91±1.32	14.06±0.67
Allium cameleon 150 mg/kg	13.25±1.10^{c}	14.36±1.71^{c}	15.02±1.36
Allium cameleon 200 mg/kg	14.96±1.50^{c}	15.86±1.61^{c}	16.89±1.06^{b}
Sildenafil 60 mg/kg	13.76±0.94^{c}	14.07±0.94^{c}	15.16±0.85

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, $^{b}P<0.01$, $^{c}P<0.001$, significantly different compared to the negative control group. FSH = Follicle Stimulating Hormone, LH = Luteinizing Hormone.

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

Results of figure 1A show a dose dependent increase of Nitric oxide in penile tissues of *Allium cameleon* treated rat varying from 10.63±0.68 ng/ml for the negative control to 15.54±1.57 ng/ml and 15.01±0.93 mM respectively at 200 mg plant extract/kg and sildefil citrate at 60 mg/kg in treated rats. A significant [F (4, 25) = 25.37, p<0.001] variation of Nitric oxide concentration was observed between treated animal and negative control ones. Concerning cGMP, aqueous extract of *Allium cameleon* also revealed a dose dependent increase of cGMP in penile tissues varying from 12.65±1.06 ng/ml for the negative control to 14.25±0.17 ng/ml and 14.81±0.55 mM, respectively at 200 mg plant extract /kg and sildefil citrate at 60 mg/kg in treated rats (Fig. 1B). cGMP concentration was significantly [F (4, 25) = 2.80, p<0.05] greater in 200 mg plant extract /kg and Sildefil citrate at 60 mg/kg treated rats than in other concentrations of plant extract and negative control treated animals compared to negative control.



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, $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, significantly different compared to distilled water-treated group. DW, distilled water; Ac100, 100 mg/kg Allium cameleon aqueous extracts

Figure 1 Effects of *Allium cameleon* aqueous extracts on penile level of nitric oxide (A) and cGMP concentration (B) of male rats after daily treatment for 30 days

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

The effects of *Allium cameleon* on malondialdehyde and reduce glutathione levels, superoxide dismutase and catalase activities of male rats after 30 days of treatments are reported in Table 6. A decrease level of MDA range significantly from 1.70±0.02 (mol/g for distilled water treated group to 1.42±0.21 mol/g and 1.12±0.06 mol/g for the treated group animals with 200 mg extract/kg and Sildenafil citrate at 60 mg/kg respectively. Post hoc analysis showed a significant difference between treatments [F (5, 28) = 26.55, p<0.001]. In contrast, superoxide dismutase activities increased from 1.14±0.06 U/g the for negative control to 2.82±0.04 U/g for *Allium cameleon* at dose 200 mg extract/kg and 3.79±0.58 U/g for the positive control treated with sildenafil citrate at 60 mg/kg. SOD concentration was significantly higher [F (5,

28) = 80.20, p<0.001] in 200 mg extract/kg and Sildenafil citrate at 60 mg/kg treated animal than in other treated groups. Captivatingly, catalase activities also increased from 88.68±2.59 mM/min/g for negative control to 109.57±3.03 and 113.47±5.46 mM/min/g respectively for the group treated with 200 mg/kg of the plant extract and sildenafil citrate treated group. Catalase activities differed significantly [F (5, 28) = 15.23, p<0.001] between animal treated groups as compared to distilled water treated group. In addition, the reduced glutathione level was increased by both *Allium cameleon* extract and sildenafil citrate, fluctuating from 2.37±0.16 mol/g for distilled water treated group to 2.86±0.27 and 3.03±0.15 mol/g respectively for the group treated with dose 200 mg extract/kg of the plant and sildenafil citrate at 60 mg/kg. Significant variation [F (5, 28) = 5.62, p<0.01] were observed in from treated animal group samples, as compared to negative control treated with distilled water.

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

Treatments	***In vivo* Oxidative stress parameters**			
	MDA (mol/g)	**SOD (U/g)**	**Catalase (mM/min/g)**	**GSH (mol/g)**
Distilled water 10 ml/kg	1.70±0.02	1.14±0.06	88.68±2.59	2.37±0.16
Allium cameleon 100 mg/kg	1.61±0.08	2.78±0.05[c]	102.76±1.91[b]	2.71±0.19
Allium cameleon 150 mg/kg	1.42±0.21[c]	2.82±0.04[c]	109.57±3.03[c]	2.86±0.27[a]
Allium cameleon 200 mg/kg	1.12±0.06[c]	3.79±0.58[c]	113.47±5.46[c]	3.03±0.15[c]
Sildenafil 60 mg/kg	1.37±0.07[c]	3.01±0.10[c]	102.73±11.24[b]	3.05±0.51[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, [b]P<0.01, [c]P<0.001, significantly different compared to the negative control group. MDA= malondialdehyde, SOD= superoxide dismutase, GSH = reduced glutathione.

4. Discussion

Plants have been used worldwide from various cultures as a safe natural source of drugs for the treatment of sexual dysfunction [20, 21]. The use of an animal model for early screening to determine the aphrodisiac potential of a drug has been recognized model [22, 23]. Additionally, the use of rat models to mimic and understand the possible effects of consumed elements is fast and simple [23]. This model is also used to evaluate aphrodisiac and stimulant activity on penile erection in case of erectile dysfunction [23]. Sexual behavior parameters like mount, intromission and ejaculatory latencies and frequencies are considered as indicators of sexual vigor, libido and potency [24]. In the present study, the administration of *Allium cameleon* extract has increased sexual behavior of model animals such as ejaculatory latency, mount, intromission, and ejaculation and erection frequencies, indicating increased excitement of male rats. A decrease of mount and intromission latencies and post ejaculatory interval were also observed, as the result of arousal in male rats. These results reflect improved performance, motivation, vigor, and high degree of sexual stimulation, thus corroborating with previous findings by other authors [22-25], confirming the enchased sexual function of male rats and suggesting aphrodisiac activity of *Allium cameleon.*

Observations in the present study could be a repercussion of the effects of the bioactive compounds at the peripheral level by direct stimulation of the cavernous body to produce Nitric oxide, a key molecule responsible for keeping the erection and extending the copulatory activity [26]. Subsequently, synthesized and secreted testosterone by the Leydig cells of the testis under the stimulation of Luteinizing Hormone (a gonadotrophin), is the main hormones contributing to the initiation and modulation of sexual behavior in rats [27,28]. The pro-sexual effects of *Allium cameleon* extract observed in this study could be attributed to the activation of testosterone production by Leydig cells. To confirm this hypothesis, the evaluations of serum testosterone, Luteinizing Hormone and Follicle Stimulating Hormone were conducted. It appears from investigation that administration of plant extract, significantly improved serum concentration of studied hormones. Similar to our findings, pretreatment of animals with *Tribulus terrestris* and *Mucuna pruriens* alone and in combination was found to increase testosterone, FSH, and LH concentrations [29,30]. Comparable results have been reported on sexual functioning with different compounds, demonstrating increase of FSH, LH, and testosterone levels [31,32]. At dose 150 mg/kg plasma level of testosterone was significantly increased compared to distilled water treated group, but the LH level was less significant, suggesting that some constituents of *Allium cameleon* could be able to mimic the role of LH and stimulate Leydig cells to produce testosterone.

The reproductive and accessory organs weights were significantly increased for all doses plant. Testosterone has been testified to be useful for the development and maintenance of testes, and ultimately, the biochemical process of sperm production [33]. The enhanced organs weight could be attributed to the response of testosterone activity on them. The improvements in the weights of accessory sexual organs of male rats are habitually associated to androgenic activity and anabolic function. Androgens can stimulate the development of testis, seminal vesicles and prostate and increase their weights [34]. Increased testis weight could indicate the high spermatic production due to increase in length of seminiferous tubules and proliferation of germ cell [35]. According to Luo and collaborators [36] all drugs or natural compounds that can increase the weights of accessory sexual organs are considered to possess androgenic properties. The increased weight of these organs would improve the motility, concentration, and decrease the anomalies of spermatozoa, which are associated with a possible high fertility rate [37]. The study of spermatogenesis shows an increase of the number of sperm in the testes. The results also indicated a significant dose dependent increase in the sperm morphology, sperm motility and sperm viability of the rats treated with *Allium cameleon* extract suggesting that the extract possess spermatogenic properties. The effect of plant or its bioactive compounds on the spermatozoa characteristics is to be mediated through the hypothalamic-pituitary-testicular axis of the animal. The increase in sperm count, viability, morphology and motility in the plant-treated rats might be due to the effect of the extract on the maturation of the spermatozoa, as well as the androgen enhancing effect of *Allium cameleon*. The extract may also have facilitated the spermatogenesis, as well as helping the functioning of sexual organs [38, 39].

One of the roles of testosterone is also the regulation of endothelial and vascular functions in the penis. It prevents vascular transformation through inhibition of vascular oxidative stress. Testosterone also leads to vasodilatation through initiation of nitric oxide production and by improving endothelial nitric oxide synthase expression [40]. The Nitric oxide (NO) and cGPM reveals a significant increase in penile tissues. The Nitric oxide increased in the penis of *Allium cameleon* treated rats might explain the increase in corpora cavernosa observed in the treated groups. Blood flow stimuli that occur at the onset of erection coordinate the process, spreading sinusoidal spaces by increasing NO release, which in turn activates guanosine cyclase, increasing the level of cyclic guanosine monophosphate (cGMP), producing muscle relaxation and vasodilation [41] leading to erection. Aphrodisiacs are substances that can stimulate libido [42]. These substances stimulate the hypothalamus to release nitric oxide, which ultimately dilates the blood vessels of corpus carvenosum and initiates the conversion of guanosine triphosphate into cyclic guanosine monophosphate under the influence of the enzyme guanylate cyclase. cGMP that increases the flux of blood into penile tissue results into penis erection [43]. The erection ends though hydrolyzation of cGMP by phosphodiesterase type-5 enzyme (PDE-5) into inactive GMP. Thus, inhibitors of this enzyme, which prevent the degradation of cGMP are used to maintain the erection longer [44].

In our finding, administrations of *Alluim cameleon* significantly increase activities of SOD and catalase, level of reduced glutathione and decrease MDA level. The implication of oxidative stress in the etiology of male infertility has been clearly recognized [45]. High level of reactive oxygen species is linked with loss of sperm motility, decrease in sperm oocyte fusion, can also cause lesion in DNA, leading to various types of mutations, imperfect spermatogenesis and causes of gene deletion leading to infertility [46, 47]. It has been reported that cell initiates defense against unwarranted free radicals by their preventative mechanisms such as antioxidant defenses [48]. Antioxidants help to defend tissues and organs such as testis, epididymis and seminal vesicles from oxidative stress [49]. Consequently, the observed results in rats treated with *Allium cameleon* could in part be due to the antioxidant property of the aqueous extract. It has been reported that an enhancement of sperm parameters after antioxidant consumption, such as sperm concentration, motility, or decrease of DNA damaged [50]. Therefore, several studies have shown reduced antioxidant defense related to alteration of sperm morphology, low sperm motility and concentration, an increase of abnormal spermatozoa [51]. Hence, increased sexual parameters and spermatogenesis might be the consequences of elevated expression antioxidant enzymes induced by *Allium cameleon*.

5. Conclusion

It appears from this research that oral administration of *Alluim cameleon* extract results in boosting sexual hormones production, improving antioxidant synthesis, enhancing sexual behaviors and increasing spermatogenesis. It has clearly revealed that bioactive compounds in the extract are capable of coping with infertility problems suggesting that the phytochemicals of the plant endorsed are better aphrodisiac alternative.

Compliance with ethical standards

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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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