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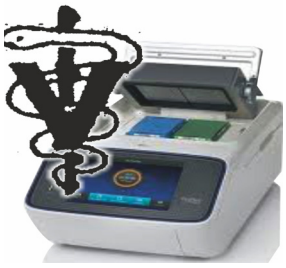

Testicular anti-inflammatory and antioxidant activity and the Reproductive enhancing potentials of ethanol extract of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae) leave in male Wistar rats.

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Abstract

Testicular inflammation and oxidative stress have contributed immensely to altering sperm qualities and played major roles in the pathophysiology of idiopathic male infertility. The use of herbal medicine in ameliorating inflammation and oxidative stress-induced damage to testicular tissues has continued to attract attention. Alchornea cordifolia has been hypothesized to possess fertility-enhancing properties. This study investigated the effects of ethanol leaf extract of Alchornea cordifolia (ELEAC) on testicular pro-inflammatory ytokines, (IL-1β, IL-2, IL-6, TNF, and IFN-γ, oxidative stress markers (MDA), ntioxidant enzymes (SOD, CAT, and GPx), and male reproductive parameters. This study demonstrated a significant ($p<0.05$) reduction in testicular IL-1β at 200 and 400 mg/kg of ELEAC exposed groups compared to the control group. Oxidative stress marker, i.e. testicular malondialdehyde (MDA) was significantly ($p<0.05$) decreased in the 200 mg/kg extract exposed group compared with the control. Testicular Superoxide dismutase (SOD), was significantly ($p<0.001$) reduced in all treated exposed groups compared with control while Catalase (CAT) and testicular Glutathione (GSH) levels were significantly ($p<0.05$) reduced in 200 mg/kg treated group compared with the control. Catalase, Glutathione, and Glutathione peroxidase activities were reduced at 400 and 800 mg/kg, however, these were not statistically significant ($p>0.05$). Analysis of male hormones revealed a significant ($p<0.05$) increase in luteinizing hormone and a reduction in serum testosterone level significantly ($p<0.05$). There was no significant ($p>0.05$) changes in the values of sperm parameters analyzed in this study. In conclusion, ethanol leaf extract of Alchornea cordifolia possesses anti-inflammatory and antioxidant properties that could be harnessed to enhancing male fertility by protecting sperm cells from damage caused by free radicals and inflammatory mediators in both acute and chronic inflammatory and oxidative stress-induced disease conditions.

Keywords: Antioxidants, *Alchornea cordifolia*, Cytokines, Inflammation, Oxidative stress, Sperm parameters,

Introduction.

The world has continued to record increased cases of male infertility, with over 50% of childlessness in marriages worldwide being related to the inability of the male partner to perform his sexual functions that may result in conception and eventual delivery of the a child. It can also be associated with failure of the male partner to produce healthy and active spermatozoa required for effective and efficient fertilization of the mature ovum [1]. That is often manifested in the form of low sperm count, low-quality sperm concentrations, and alterations in seminal DNA integrity and morphology in one sample out of every two sperm analyses carried out between 1 and 4 weeks apart [2]. Several factors have been linked with male infertility including; hormonal disorder, environmental or physical factors, lifestyle adapted by the individuals, poor testicular development and hash testicular microenvironment, nutritional factors, and psychological disturbances. Apart from these factors, elevation in reactive oxygen species (ROS) productions has been shown to play a very vital role in the pathophysiology of idiopathic male infertility because of its capacity to damage the integrity of DNA, enhance sperm plasma membrane fluidity and accelerated lipid peroxidation reaction with a resultant dysfunctional spermatogenesis [3]. Though the optimal performance of spermatozoa requires that these substance be produced; especially to enhance sperm cell capacitation and improved reproductive functions, sustenance of sperm viability, growth, motility and maturation, as well as contributing to the stability of DNA by facilitating the establishment of strong disulfide bond require for maintenance of chromatin structure and the transportation of sperm cells within the female oviduct through the liquefaction and coagulation of seminal fluid; however, when produced in excess, ROS may deplete the available antioxidant enzyme and non-enzymatic antioxidant system, with a resultant destruction of the cellular membrane of the spermatozoa [4]. Report has shown that increased seminal fluid's reactive oxygen species (ROS) could be linked to venous stasis, occasioned by varicoceles, ischemia, hypoxia, alteration in circulatory activity, and infections; interestingly, there is a complex interrelationship between cytokines, hormones and other oxidative stress mediators that influence and promote the synthesis and release of free radicals into testicular microenvironment [5], most of which are characterized by inflammatory reactions.

According to Alshahran *et al.* [6], high levels of pro-inflammatory cytokines are often associated with inflammatory induced diseases caused by pathogenic microorganisms in male organism especially in bacterial induced prostatitis, which may stimulate the generation of ROS that may cause damage of sperm cells' DNA by apoptosis. Testicular cytokines (IL-1β, IL-2, IL-6, TNFα, and IFN-γ are produced by Leydig and Sertoli cells, macrophages, and other leukocytes that infiltrate the testicular environment during acute or chronic inflammatory episodes. Based on the report of Wu *et al.* [7], elevated testicular TNFα, IL-1β and IL-6 have been associated with decrease in sperm count, motility, and morphology. These are the abnormalities often seen in infertile men. Furthermore, oxidative stress-induced inflammatory mediators, increased lipid peroxidation (malondialdehyde), and ROS accounts for most of the damages observed with seminal samples [6]. These changes also induce apoptosis through sustained proliferation and differentiation of T-cells and natural killer cells.

Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione S-transferase (GST) are the major antioxidant enzyme system, which works in synergy with the non-enzymatic antioxidant, glutathione (GSH), vitamin C, taurine, vitamin E and pyruvate that are responsible for scavenging harmful excess ROS and products of lipid peroxidation, nitroxyl radical and carbonyl compounds, from the testicular environment to minimize tissue damage and death. The antioxidant acts as shield that prevent the devastating and deteriorating effects of these substances and the maintenance of reproductive efficiencies through repairs and the preservation of the sperm DNA integrity [8]. The GPx is considered the most important of the enzymes in the testes that enhance male fertili-

ty. Based on the recommendations of the world health organization, the use of some medicinal plants/herbs and their derivatives has become an acceptable fertility-enhancing product, particularly in developing countries of the world [9]. Phytochemical constituents such as flavonoids and polyphenols have been shown to possess anti-inflammatory and antioxidant activities and are readily employed in herbal medicine for the management of different disease conditions including reproductive dysfunctions.

Alchornea cordifolia, is a bushy perennial shrub or small tree, measuring about 8-10 meters high, found widely distributed around tropical African forests, and located near water or wetland [10]. Several medicinal and pharmacological activities of the plant, including its antifungal, antibacterial, and spasmolytic activities have been investigated. The anti-inflammatory, antioxidants and hepatoprotective activities of the plant have been reported [11; 12]. However, there is limited information on the activity of ethanol extract of *A. cordifolia* leaves on testicular antioxidants, pro-inflammatory cytokines, sperm parameters, and apoptosis in animal tissues. Therefore, the present study was designed to investigate the testicular anti-inflammatory, antioxidants and reproductive enhancing potentials of ethanol extract of *A. cordifolia* leaves in male albino rats.

Materials and methods

Collection of plant material and identification

The leaves of *Alchornea cordifolia* were harvested from Aikplia village in Ugbokolo, Okpokwu Local Government Area of Benue State, Nigeria. It was identified and authenticated by Dr. Okoh Thomas and the voucher number (FUAM/BOT/HERB/02781) was deposited at the herbarium of the Department of Botany, Faculty of Science, Federal University of Agriculture Makurdi Benue State, Nigeria for reference purposes.

Extract preparation

The leaves were harvested and washed in running tap water to remove sand and other solid particles, dried under laboratory conditions and pulverized using an electric blender. The leaves were extracted according to a previous protocol, as reported by Ejeh *et al.* [13]. Thereafter, the filtrate was evaporated to dryness using a water bath at 35 ^{o}C to obtain a yellow-brown residue.

Experimental design

Twenty adult male albino rats weighing between 200-250g were sourced from the animal house in the Department of Veterinary Physiology and Biochemistry, University of Abuja. The rats were acclimatized for a week prior to the commencement of the study. The rats were grouped into four groups of five rats each and kept in a plastic cage maintained at the standard condition of 12/12 hour light/dark cycle, and fed commercial pelletized feed (Grand Cereal, LTD, Jos Nigeria) and clean water provided *ad libitum* under controlled temperature and humidity. Ethical clearance was obtained from the University of Abuja Ethics Committee on Animal Use (UAECAU) with approval number UAECAU/2022/005. The acute toxicity study of the extract was conducted in a previous study and the extract was found to be safe up to 1000 mg/kg in rats.

Group I, animals received distilled water

Group II animals received 200 mg/kg of ethanol leaf extract

Group III animals received 400 mg/kg of ethanol leaf extract and

Group IV was administered 800 mg/kg ethanol leaf extract

The rats were administered the extract daily orally for four weeks. Following the last administration, the animals were sacrificed, the right testes exteriorized and cleaned with normal saline before they were used to evaluate the various anti-inflammatory and antioxidants parameters.

Preparation of testicular tissue homogenate

The left testis obtained from each rat in the groups was homogenized and aliquoted with 10 ml of 1100 mM potassium phosphate buffer containing 1 mM EDTA pH 7.4 after it was freshly exteriorized from the scrotal sac. Centrifugation of the homogenates was done for 30 minutes at

1200 g at 4°C. The supernatant was collected, and used for further investigations. The total amount of soluble protein in tissue homogenates of testes was determined using crystalline BSA as standard.

Measurement of testicular pro-inflammatory activity

An enzyme-linked immunosorbent assay (ELISA) kit for the markers (TNF-α, IL-1β, IL-2, IL-6, and IFN-γ) were purchased from Proteintech, Wuhan Sanying, China, and used for the quantitative determination of testicular IL-1β, IL-2, IL-6, TNFα, and INF-γ) using ELISA reader (Perkin Elma, USA).

Briefly, 100 ml of samples and standards were added into the wells already pre-coated with antibodies specific for IL-1β, IL-2, INF-γ, IL-6, and TNF-α and incubated for 5 hours at 37. Unbound substances were removed and 100 ml of biotin-conjugated antibody specific for each of IL-1β, IL-2, INF-γ, IL-6, and TNF-α were added to each of the wells. After washing, 100 ml of avidin-conjugated Horseradish peroxidase (HRP) was added to the wells and incubated for 3 hours at 37, followed by the addition of 100 ml of 3,3,5,5-tetramethylbenzidine (TMB)TMB substrate solution. It was incubated again for 1 hour at 37 to give a colour in proportion to the concentration of IL-1β, IL-2, INF-γ, IL-6, and TNF-α bound in the initial step. The plates were gently shaken to enhance thorough mixing and the color intensity was measured at 470 nm for IL-6, 488 nm for TNF-alpha, and 450 nm for IL-2, INF-γ and IL-1β respectively.

Determination of catalase activity

Determination of catalase (CAT) activity was based on the process which depends on the decomposition of H_2O_2 (hydrogen peroxide). The reaction mixture was prepared by the addition of 100 ml of 7.0 mM H_2O_2, 750 µl of 50 mM potassium phosphate at the pH of 5.0, and 30 µl of tissue homogenates. The disappearance of H_2O_2 by the action of CAT was measured in the reaction mixture at 240 nm by spectrophotometric technique.

Determination of Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined by Kakkar *et al.*[14] method. Tissue homogenates were first centrifuged at 1500 g for 10 minutes, then for 15 minutes at 10000 g. From the supernatant obtained, 150 µl was added to the reaction mixture containing 59 µl of 190 Mm phenazine methosulphate and 609 µl of 0.05 mM sodium pyrophosphate buffer of pH 7.0. To start the reaction, 100 µl of 800 mM NADH was added, then 600 µl of glacial acetic acid was added after 1 min to stop the reaction mixture, and the absorbance was read at 560 nm (unit/mg protein) with a spectrophotometer.

Testicular Tissue Malondialdehyde Determination

Malondialdehyde (MDA) levels were determined in the supernatant of testicular tissue homogenate using thiobarbituric acid (TBA) reaction following the method of Ohkawa *et al.* [15]. To 1 mL of supernatant, 0.5 mL of 30% trichloroacetic acid (TCA) was added, followed by 0.5 mL of 0.8% TBA. The tubes were kept in a water bath for 30 minutes at 80 °C. After 30 minutes of incubation, the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800 g for 15 min. The absorbance of the supernatant was read at 540 nm at room temperature against an appropriate blank. MDA concentration was measured from the standard calibration curve. Lipid peroxidation was expressed as nanomoles of MDA per milligram of protein.

Determination of Testicular Glutathione Peroxidase Activity

The method by Mohandas *et al.* [16], was adopted, while NADPH was used as a substrate to evaluate testicular glutathione peroxidase (GPx) activity. The reaction mixture was constituted by the addition of 60 µl of 1mM sodium azide, 60 µl of 1mM EDTA, 30µl of glutathione reductase, 30 µl of 1mM GSH, 10 µl of 0.25 mM H_2O_2

and 800 µl of 0.1 M sodium phosphate buffer, pH of 7.4. Then, 60 µl of tissue homogenates were mixed with the constituted reagent. To initiate the reaction, 50 µl of 0.2 mM NADPH was added and incubated at 37 ^{o}C for 5 minutes. Absorbance was measured at 340 nm to determine the disappearance of NADPH, and the value was recorded as NADPH oxidized per minute per mg of protein. The blank tube containing only distilled water was run alongside.

Determination of Testicular Glutathione Activity

The method described by Trent and Lynette, [17] was followed to measure reduced glutathione (GSH) levels in the testicular tissue samples. Briefly, 500 µl of testes supernatant was mixed with 500 µl of sulfosalicylic acid (4%) to carry out the precipitate. The reaction mixture was incubated for 1 h at 4 and then centrifuged at 1200g for 20 minutes. The supernatant was collected and 50 µl of it was added to the reaction mixture containing 60 µl of 100 mM 5, 5'-dithio-bis' 2-nitro benzoic acid (DTNB), and 900 µl of 0.1M K_2PO_4-potassium phosphate buffer at pH 4. The yellow-coloured complex was formed due to the reaction of reduced glutathione with DTNB. The absorbance was immediately read at 412 nm.

Evaluation of the effects of ethanol leaf extract on Reproductive Parameters of male Wistar rats sperm motility

Sperm motility was evaluated using the swim-out technique. After exteriorization and trimming of the cauda epididymis to remove fat and tissue debris. A small incision was made through it and then placed in 1 ml of normal saline for a few minutes to allow the sperm cells to swim into the fluid. Thereafter, a drop of the suspension was placed on a grease-free slide, a cover slip was placed on it, and viewed at 400 magnification. Individual sperm motility was determined by counting progressive motile sperm across different fields per unit area. The values were presented as percentage of motile spermatozoa

Evaluation of sperm viability

The method of Blom [18] was used to assess sperm viability. Briefly, a drop of sperm suspension was mixed with a drop of eosin-nigrosin stain on grease-free microscope slides and allowed to stand for a few seconds. Thereafter, a smear was made, air-dried, and viewed at x_{40} magnification. Two hundred spermatozoa were counted across the different fields and the percentage of live spermatozoa were recorded and tabulated

Evaluation of epididymal sperm concentration

The method of Robb *et al.* [19] was used in the evaluation of epididymal sperm concentration. Briefly, a drop of sperm suspension prepared in 0.05% formol-saline was placed on a pre-warmed improved hemocytometer and counted to assess epididymal sperm concentration. Thereafter, the morphometric parameter such as testicular weight, and length of the epididymis, including its weight was measured with a standard weighing balance, and ruler while testicular volume was evaluated by applying the principle of floatation.

Effects of Ethanol Leaves Extract of *A. cordifolia* on male hormones

Male hormones i.e. testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were evaluated in this experiment using commercial ELISA kits obtained from Monobind Inc. (Lake Forest, CA 92630, USA) following the manufacturer's instruction and the values were obtained in triplicated and the concentration extrapolated from a plotted concentration versus absorbance curve.

Statistical analysis

Data obtained from this study were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was employed to analyze the statistics while the level of significance between the treated groups and the control group was assessed using Dunnett's test at $P<0.05$.

Results

The effects of ethanol leaf extract of *Alchornea cordifolia* on testicular tissue pro-inflammatory cytokines (IL-1β, IL-2, IL-6, TNFα, and IFN-γ revealed a significant ($p<0.01$) reduction in the testicular levels of IL-1β and IL-2 in the group exposed to 200 mg/kg, and a significant ($p<0.03$) reduction in IL-1β in the group treated with 400 mg/kg when compared with the normal control group. No significant ($p>0.01$) difference in the testicular levels of IL-6, TNF-α and IFN-γ at all the doses used (200, 400, and 800 mg/kg) in comparison with the normal control. The reduction in the levels of IL-2 at 400 and 800 mg/kg was found to be statistically insignificant ($p>0.05$) in comparison to the control (Table 1).

Table 1. Effect of ethanol extract of *Alchornea cordifolia* leaves on pro-inflammatory cytokines in testicular tissues of Wistar rats

Groups	IL-1β (pg/ml)	IL-2 (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)	IFN-γ (pg/ml)
Control	130.4±0.55	293.1±10.72	210.6±1.70	0.77±0.03	0.97±0.03
200 mg/kg	101.7±1.30*	210.5±16.32**	200.0±2.67	0.89±0.02	1.02±0.02
400 mg/kg	106.1±8.50*	220.0±27.22**		0.85±0.05	0.95±0.01
800 mg/kg	124.2±7.21	257.3±20.94		0.75±0.06	1.00±0.03

Key: n =5; *, **= significant ($p<0.05$, 0.01) decrease compared to the control

Effect of ethanol extract of *Alchornea cordifolia* leaves on antioxidant enzymes in testicular tissues of Wistar rats.

There was a significant ($p<0.0001$) decrease in the levels of testicular tissue malondialdehyde (MDA), a marker of lipid peroxidation in the group treated with 200 mg/kg, and a significant ($p<0.001$) reduction in testicular superoxide dismutase (SOD), significant ($p<0.01$) decrease in catalase and reduced glutathione (GSH) levels of the same group treated with a dose of 200 mg/kg in comparison with the normal control group. The measured SOD activity was significantly decreased in 400 and 800 mg/kg treated groups, whereas the catalase and glutathione levels were not significantly ($p>0.01$) altered at the 400 and 800 mg/kg doses used compared with the control group (Table 2).

Table 2 Effect of ethanol extract of *Alchornea cordifolia* leaves on antioxidant enzymes in testicular tissues of Wistar rats

Groups	MDA (nmol/mg protein	SOD (µmol/min/mg protein)	CATALASE (µmol/min/mg protein)	GSH (µmol/min/mg	GPx (µmol/min/mg protein)
Control	5.44±0.12	9.95 ± 0.38	10.62 ± 0.42	41.0.8 ± 0.20	4.94 ± 0.96
200 mg/kg ACE	3.91 ± 0.01*	7.60 ± 0.08*	9.45 ± 0.02*	36.91 ± 14*	3.83 ± 0.02
400 mg/kg ACE	4.92 ± 0.11	8.24 ± 0.48*	9.96 ± 0.18	37.91 ± 1.46	3.97 ± 0.09
800 mg/kg ACE	4.59 ± 0.28	8.22 ± 0.27*	9.68 ± 0.11	38.09 ± 0.69	3.91 ± 0.03

Keys: n=5; *= significant decrease compared to control at $p<0.05$. ACE = *Alchornea cordifolia* extract

Effects of Ethanol Leaf Extract of *A. cordifolia* on Male Reproductive Hormones

Analysis of male reproductive hormones is presented in Table 3. It was observed that both serum LH and FSH concentrations were significantly (P>0.05) increased at the various extract doses used compared to the control. However, serum testosterone concentrations were decreased significantly (P>0.0001) at 200 and 400 mg/kg treated groups compared with the control. Though there was a similar decrease in the 800 mg/kg treated group compared with the control, however, this was not significantly (p>0.05) different compared to the control.

Table 3. The effects of ethanol leaf extract of *A. cordifolia* on male reproductive hormones

Groups	LH (pg/ml)	FSH (ng/ml)	TESTOSTERONE (pg/ml)
Control	2.0±0.2	8.2±1.07	5.4±0.39
200 mg/kg	3.6±0.19*	13.24±1.70	2.39±0.24††
400 mg/kg	4.48±0.20*	16.84±2.3**	1.88±0.46††
800 mg/kg	3.62±0.33*	10.18±0.71	4.3±0.22

Key: n=5; * = significant increase at p<0.05 compared to control; ** = significant increase at p<0.01 compared to control; †† = significant decrease at p<0.05 compared to control

v

4.1.4. Effects of ethanol leaf extract of *Alchornea cordifolia* on sperm parameters.

Improved sperm quality is a measure of the reproductive capacity of every male organism. To better appreciate the effects of ethanol leaf extract of *Alchornea cordifolia* on the male reproductive indexes, sperm parameters such as extrapyramidal sperm count, motility, viability, concentration and percentage abnormality were investigated. Table 4.0. Shows the results of those parameters measured. It was observed that there were significant (p<0.05) increases in epididymal sperm count in the extract-exposed groups compared with the control, the percentage of sperm viability, and motility were also increased across the extract-exposed groups but decreased in groups exposed to 400 and 800mg/kg respectively. The increase noticed was not dose-dependent. Though these parameters were observed to have increased in the extract treatment groups compared with the control however, the values were not significant (P< 0.05) statistically.

Table 4. The effects of ethanol leaf extract of *A. cordifolia* on reproductive parameters

Parameters	Control	200 mg/kg	400 mg/kg	800 mg/kg
Sperm motility (%)	85.60±2.1	93.40±1.6	82.20±1.5	71.00±1.0
Sperm Viability (%)	89.50±1.6	92.20±2.3	91.20±1.1	86.25±5.7
% Abnormality	3.25±1.1	2.80±0.8	4.00±1.3	5.00±1.8
Test. sperm count ($X10^6$/ml)	200.04±43.6	140.00±6.0	108.80±13.0	159.00±31.7
Epid. Sperm count ($X10^6$/ml)	60.25±12.4	86.30±14.1	65.25±11.1	98.95±16.2
Testes weight (g)	1.46±0.1	1.31±0.0	1.61±0.1	1.61±0.0
Testes volume (ml)	1.28±0.1	1.42±0.2	1.68±0.3	2.12±0.8
Epididymal weight (g)	0.37±01	0.38±0.1	0.42±0.1	0.42±0.0
Epididymal length (m)	5.04±0.1	4.50±0.1	5.17±0.1	3.8±0.7

The values presented are mean ± SEM., n=5; Test=Testicular; Epid. = Epididymal.

Discussion

vHerbal medicine has shown genuine promise in the management of male infertility both in developed and developing countries of the world [20]. The exploration of herbs as remedies for reproductive dysfunctions has been attributed to the presence of bioactive compounds with potent antioxidants and anti-inflammatory properties [21]. There is an increasing interest in antioxidants and anti-inflammatory agents, especially those with the capacity to scavenge free radicals and prevent their damaging effects on animal tissues and cellular components but devoid of adverse effects [11]. In this study, we evaluated the effects of ethanol leaf extract of *A. cordifolia* on testicular oxidative stress/ antioxidant enzymes, as well as on pro-inflammatory cytokines, sperm parameters, and male hormonal levels. Presence of inflammatory mediators and reactive oxygen species are known to adversely influence reproductive functions by disrupting gonadal steriodogenic activity with resultant reduction in spermatogenic processes. In this study, we recorded a significant ($p< 0.05$) reduction of testicular IL-1 and IL-2 in group exposed to 200 mg/kg of the extract relative to the control group that received distilled water. According to Wu *et al.* [7], increased seminal IL-1β, IL-2, and TNFα have been implicated in low sperm count, motility, and poor sperm qualities, with subsequent male infertility. Significant ($p< 0.05$) reduction in the levels of IL-1β and IL-2 at the dose of 200 mg/kg may suggest a strong anti-inflammatory activity of this plant extract in animals. These findings corroborate earlier findings of Manga *et al.* [21] who reported inflammatory reducing activity of methanol leaves extract of *A. cordifolia* in mice, stating further that its anti-inflammatory activity is concentration-dependent, contrary to our observation in which the highest anti-inflammatory activity was recorded in the 200 mg/kg dose used. The anti-inflammatory activity recorded in this study may be attributed to the presence of polyphenols and tannins seen in the phytochemical evaluation [13], and the work of other authors [21; 11]. IL-1β is an immune-derived cytokine and its secretion is promoted under ischemia and hypoxic conditions, hence, an elevated concentration of IL-1β induces detrimental effects on the testes of infertile males with varicocele [5]. The ability of this cytokine to induce and maintain inflammations in the testes was reduced as demonstrated in this study. This may further suggest the potential therapeutic activity of this plant in some inflammatory conditions associated with male infertility in future.

Furthermore, there was a significant reduction in the level of testicular malondialdehyde (MDA), following the treatment of rats with 200 mg/kg b. wt. of *A. cordifolia* leaf extract may be an indication of its ability to scavenge and mop up free radicals from the testicular environment. The MDA is a product of lipid peroxidation and a marker of oxidative stress in animals and man. The significant ($p<0.05$) decrease in the levels of testicular SOD, GSH, and catalase may be associated with exhaustion as a result of constant production of the reactives radicals and the consequent scavenging actions. The results may also be linked to the impact of heavy metals in the environment where the plant was harvested [13]. According to Elmallah *et al.* [22], the exposure of rats to cadmium chloride could result in decreased testicular antioxidant markers such as SOD, catalase, glutathione peroxidase, and glutathione reductase. However, in this study, the anti-inflammatory and antioxidant activity recorded may be linked to the presence of phenol, flavonoid, and saponin in the ethanol leaf extract of *A. cordifolia* [11]. These phytochemicals are also known to possess hypothalamic-pituitary-gonadal axis (HPA) stimulating capacity [23]. Gonadotropins such as Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) are the main hormones associated with the regulation of male reproductive functions and behaviour [23] and it has been shown that increase oxidative stress induced by pro-inflammatory cytokines such as IL-1β, and IL-6 could adversely alter the hypothalamic-pituitary-gonadal axis (HPG) activity with decrease testosterone secretion [24]. Hence, a disruption of the regulatory activity of HPA may be the primary cause of male infertility [25]. The outcome of our study also revealed a significant ($p<0.05$) in-

crease in serum LH across the treatment groups compared with the control and a significant ($p<0.05$) increase in serum FSH concentration in the 400mg/kg treated group compared with the control. This suggests that the plant extract may possess androgenic activity which may be linked to its ability to reduce the level of IL-1β as seen above. However, compared to the control, a significant ($p<0.05$) reduction in serum testosterone was observed in 200 and 400 mg/kg body weight treated groups. Our observation agreed with the report of Ngaha-Njila *et al.* [23], notwithstanding the disparity in the serum testosterone concentration and the testicular testosterone concentration between our study and theirs. The decrease in testosterone may be associated with the high cadmium concentration recorded in the extract as observed earlier [13]. Furthermore, it has been shown that cadmium reduces testosterone, concentration, decreases testicular enzyme activity, and alters spermatogenic activity in male rats [22]. Since increase pro-inflammatory cytokines-mediated oxidative damage have been shown to inhibit the activity of steriodogenic acute regulatory receptor protein (StAR), and a resultant suppression of androgenic hormone production and the impairment of spermatogenic processes, the reduction in the inflammatory cytokine (IL-1β, and TNF-α), marker of lipid peroxidation (MDA) and the increase gonadotropin concentration recorded in this study, may indicate the potential ability of the extract to ameliorate inflammatory conditions that could impair male reproduction and affect fertility. Intriguingly, analysis of sperm parameters did not reveal any significant changes in the treated groups compared to the control. Although, there was an increase in sperm motility, viability and epididymal count in the 200 mg/kg exposed group, these were not statistically significant but may be clinically relevant in the practical usage of the plant in enhancing male fertility. The results of the sperm parameters as recorded in this study may be attributed to the duration of exposure and the method of extraction.

Based on our knowledge, increased reactive oxygen species could trigger overwhelming pro-inflammatory cytokines, which may cause several sperm cell DNA damages, protein breakdown, and increased plasma membrane permeability with resultant apoptosis [22]. From our present findings, it may be inferred that the administration of this extract had no damaging effect on the sperm cells of male albino rats and this study has further demonstrated that ethanol leaf extract of *A. cordifolia* has anti-inflammatory and antioxidant activities in the testicular tissues of albino rats and could be used to ameliorate damages induced by oxidants and other pro-inflammatory mediators, though more works need to be done to unveil the full medicinal values of the plant.

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Conflict of interest

Authors declare no conflict of interest

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Authors' contributions.

SAE, HAA, PAO and JNA participated in the conceptualization of this work. All author were involved in data collection and analysis. SAE prepared the manuscript while HAA, PAO, JNA, COE and SEA reviewed the manuscript. Final manuscript was read by all authors.

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