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Full Length Research Article

ASSESSMENT OF *GARDENIA ERUBESCENS* AQUEOUS ROOT CRUDE EXTRACT ON MALE SEXUAL FUNCTIONS-RELATED BIOCHEMICAL PARAMETERS IN CLONIDINE-INDUCED SEXUAL DYSFUNCTION WISTAR RATS

Omoniwa Babasoji Percy[1*], Longdet Ishaya Yohanna[2], Okpatu Gabriel Chizoba[1] and Oladele Kehinde Gideon[2]

[1]Ethnopharmacology, Reproductive Biochemistry and Biochemical Toxicology Laboratory, Department of Science Laboratory Technology, University of Jos, Nigeria.

[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Jos, Nigeria.

*Corresponding Author Email Address: sojiomoniwa@gmail.com

ABSTRACT
This study evaluated the phytochemistry, male sexual function (MSF)-enhancing and toxicity potentials of aqueous root extract of *G. erubescens* (AREGE). For the MSF test, 30 rats were randomly divided into groups A, B, C, D, E and F, each group containing 5 rats. Group A represented the control and received only distilled water. Sexual dysfunction was induced in rats of groups B – F by orally administering clonidine (0.5 mg/kg body weight [BW]). Rats in groups B – F were thereafter treated with distilled water, 13.43 mg/kg BW Kongy [standard drug], AREGE [50 mg/kg BW], AREGE [100 mg/kg BW] and AREGE [200 mg/kg BW] respectively. Rats were treated for 7 days, sacrificed and their blood, penises and testes collected for analysis of selected MSF-associated biochemical parameters. For the toxicity study, 40 rats were randomly divided into groups W, X, Y and Z of 10 rats each and administered distilled water, AREGE [50 mg/kg BW], AREGE [100 mg/kg BW] and AREGE [200 mg/kg BW] respectively. Five rats per group were sacrificed 24 h after treatment for 1 day while the rest were sacrificed 24 h after treatment for 21 days. Their blood, livers and kidneys were collected for liver/kidney function indices determination. Extract contains alkaloids, flavonoids, steroids, iron, zinc, selenium, glutamic acid, arginine, phenylalanine, tryptophan and tyrosine among others. Clonidine reduced testosterone, luteinizing hormone (LH), dihydrotestosterone (DHT), nitric oxide (NO) and prolactin levels. The extract significantly increased ($p < 0.05$) testosterone, serum LH, DHT, prolactin and penile NO concentrations when compared with the clonidine and distilled water-treated group. Extract also significantly increased ($p < 0.05$) serum gammaglutamyl transferase (GGT), sodium, phosphate, creatinine, urea, liver/serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) but reduced kidney GGT and alkaline phosphatase (ALP) and serum total protein levels when compared with the control. Available results show that AREGE possesses MSF-enhancing activity but poses a threat to the normal functioning of the liver and kidney.

Keywords: *Gardenia erubescens, clonidine, sexual dysfunction, dihydrotestosterone, nitric oxide*.

INTRODUCTION
Reproduction is the biological process by which new individual organisms are produced from their parents. Reproduction is very important because it ensures propagation of species. In humans, marriage is necessary for the purpose of procreation and sexual gratification/enjoyment of both partners, through sexual intercourse. Normal sexual intercourse in males requires the sexual organs and factors relating to erection of the corpulatory organ to function normally (Yakubu and Akanji, 2011).

Sexual dysfunction is the difficulty experienced by an individual or a couple during any stage of a normal sexual activity, including physical pleasure, desire, preference, arousal or orgasm (Nolen-Hoeksema, 2014). It is an inability to achieve a normal sexual intercourse (Kotta *et al*., 2013). Male sexual dysfunction has long been known to be common, and it includes erectile dysfunction (ED), diminished libido (arousal difficulties), abnormal ejaculation, orgasmic disorder, and failure of detumescence (Kotta *et al*., 2013; Rosen and Khera, 2021). Male sexual dysfunctions therefore pose a huge challenge to procreation and also marital bliss and therefore should not be taken for granted. In recent times, the knowledge of normal male sexual function (MSF) and the causes of SD have become better understood, and effective treatments are available (Rosen and Khera, 2021). Available treatment options however have side drawbacks that may discourage adherence to treatment by sufferers. For example prosthetic devices can be used to manage ED (Le and Burnette, 2015). However, there are numerous limitations and areas for improvement which include high cost expense (devices range in price from $8,000 - $14,000), complications from surgery, risk of infection, anesthesia complications and the unnatural feel of device (Rodriguez and Pastuszak, 2017). In addition, Testosterone replacement therapy (TRT) which is a widely used treatment for men with symptomatic hypogonadism also have side effects which include exacerbation of prostate cancer, male breast cancer and polycythemia among others (Osterberg *et al*., 2014). Medicinal plants therefore provide an opportunity to obtain an effective therapy for male sexual dysfunction (MSD) that will address the drawbacks of already existing options.

The World Health Organization (WHO) defined medicinal plants as plants that contain compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful

drugs (Islam *et al*., 2012). Natural substances/plants have historically been used as aphrodisiacs in some parts of the world. An aphrodisiac is any type of food, drink or drug that is capable of causing sexual desire or arousal, increase sexual potency and increase sexual pleasure (Sabo *et al*., 2017). These natural substances are believed to exert their activity via increase in serum testosterone or a combined increase in follicle stimulating hormone and LH. They also can elicit their activities through an increased NO synthesis (Sabo *et al*., 2017). One of such plants possessing these effects is *G. erubescens* (Stapf and Hutch).

G. *erubescens* (Stapf and Hutch) belongs to the genus Gardenia of flowering plants in the coffee family called Rubiaceae. It is commonly called Cape jasmine and popularly called *Gaude* and *Dingalli* by the Hausa and Fulani people of Nigeria respectively. G. *erubescens* is widely spread in tropical Africa, from Senegal to Sudan and Uganda to Northern Nigeria and in Ubangi (Sabo *et al*., 2017; Ibrahim *et al*., 2011; Ayeni and Kayode, 2019; Korotimi *et al*., 2019). It is a shrub species with a maximum height of 3 m above the ground. The wood of the species is yellow, hard, and compact. The pale yellow, ellipsoid fruit can be 3 – 5 cm long. The leaves are opposite or in whorls of three or four, 5 – 50 cm long and 3 – 25 cm broad. They are dark green and glossy with a leathery texture. The flowers are solitary or in small clusters, either white, or pale yellow, with a tubular-based corolla with 5 – 12 lobes (petals) from 5 - 12 cm diameter (Korotimi *et al*., 2019).

Water decoctions of the aerial parts are used locally in Northern Nigeria as a multipurpose remedy for treating gonorrhoea, abdominal disorders, loss of appetite, insomnia and venereal diseases (Parmar *et al*., 2000; Ayeni and Kayode, 2019). The root decoction (taken internally) and leaves (applied externally) are used for treating syphilis and gonorrhea (Fern, 2021). The roots and fruits are commonly used as aphrodisiac among the Hausa community of Nigeria. The roots and seeds are used to treat foot and mouth disease in animals (Saganuwan, 2017). Fruits are used as an appetite suppressant in the treatment of obesity and also traditionally used against hepatitis (Pare *et al*., 2016).

In a study conducted by Ibrahim *et al.* (2011), on bioethanol production, *G. erubescens* fruits were used as substrate and the results from the study showed that *G. erubescens* fruit gave a high cumulative ethanol yield after seeding the hydrolysate with *Saccharomyces cerevisiae* for two days. Sabo *et al.* (2017) reported that experimentally, methanol extract and saponins fraction of *G. erubescens* showed sedative, analgesic, hypotensive and diuretic effects *in vivo* in rats, mice and cats and also that the aqueous stem bark and ethanol leaf extract has *in vitro* antitrypanosomal activity.

Although MSD is not a life-threatening ailment, it impacts negatively on sufferers' self-esteem and reduces overall output and productivity. As mentioned earlier, the available treatment options are not without their drawbacks which necessitates the continued search for better options. The aim of this study therefore is to ascertain the acclaimed MSF-enhancement potential of AREGE and also determine its phytochemistry and toxicity on the liver and kidney of wistar rats.

MATERIALS AND METHODS

Plant material

G. erubescens root was collected from Bintiri B, Saya, Bassa Local Government Area, Plateau State, Nigeria. It was identified and authenticated at the herbarium of the Department of Plant Science and Biotechnology, University of Jos, and voucher number JUHN20000308 was obtained following deposition of a voucher specimen.

Experimental animals

One hundred (100) Wistar rats (70 male and 30 females) of average weight 150.35 ± 25.50 g (for males) and 120.23 ± 20.20 g (for females) were obtained from the Animal House Unit of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. The rats were housed in plastic cages and allowed free access to standard rat pellet and tap water.

Ethical clearance

Wistar rats were handled according to guidelines specified by the Institutional Animal Care and Use Committee (IACUC) of University of Jos, Nigeria and the research was allocated reference number UJ/FPS/F17-00379.

Drugs, assay kits and reagents

Clonidine hydrochloride tablet (Lot No. 252440), Kongy capsule, Progesterone injection (Lot No. 170811) and Premarin tablet (Oestradiol conjugate) (Lot No. L95455) were products of Sandoz Limited, Surrey, England; HC Nature Care and Life Sciences, Lagos, Nigeria; Zhejiang Jinling Tianfeng Pharmaceutical, Huzhou City, Zhejiang, China and Pfizer limited, Kent, United Kingdom respectively.

Assay kits for DHT, ALP, phosphate and sodium were products of Elabsience Biotechnology Co., Ltd, Houston, Texas, USA. Kits for prolactin and LH were products of CalBiotech Inc., El Cajon, California, USA while that of testosterone was a product of Oxford Biomedical Research, Inc. Oxford, USA. Kits for total protein, ALT, AST, urea, potassium, creatinine and GGT were products of Fortress Diagnostics Limited, Antrim, United Kingdom. Assay kit for calcium was a product of Egyptian Company for Biotechnology, Cairo, Egypt while that of albumin was a product of Pointe Scientific, Inc. Canton, USA.

Preparation of extract

G. erubescens roots were washed, chopped to pieces and air-dried at room temperature (23.0 ± 1.5 ^{O}C) for 21 d and further oven-dried (Carbolite PF 200, Keison Products, Essex, United Kingdom) at 50 ^{O}C to constant weight. The dried roots were ground into fine powder using a hammer mill (Model PC 200 x 300, DEWO Machinery Company Ltd, Zhengzhou, Henan, China). Hot aqueous extract of *G. erubescens* root was prepared by adding 600 g of the fine root powder to 2500 mL of boiled distilled water and stirred properly with a glass rod until homogeneity was achieved. The mixture was then kept for 48 h in a refrigerator (4 ^{O}C) with intermittent stirring. The mixture was filtered using clean white muslin fabric and complemented by Whatman No1 filter paper. The filtrate was then concentrated in an oven at 50 ^{O}C and the percentage yield calculated to be 11.33 % (w/w). The extract was reconstituted in distilled water to give the required doses used in this study (Kayode and Yakubu, 2017).

Secondary metabolite analysis
The screening of *G. erubescens* sample for alkaloids, tannins, flavonoids, steroids, terpenoids and anthraquinones was done by adopting the methods described by Egbuna *et al*. (2019). The detected secondary metabolites (i.e. alkaloids, tannins, flavonoids and steroids) were quantified by the methods described by Egbuna *et al*. (2019).

Mineral and amino acid analysis
Powdered sample of *G. erubescens* aqueous root extract was analyzed for its mineral constituents using a hand held X-Ray Florescence analyser (Thermo Scientific, Niton XL3t GOLDD+, Massachusetts, USA). Amino acid analysis of *G. erubescens* aqueous root extract was carried out using the method described by Spackman *et al*. (1958).

Induction of male sexual dysfunction
Male Sexual Dysfunction was induced in mature sexually experienced male rats by a single dose oral administration of 0.5 mg/kg BW clonidine (Lot No. 252440, Sandoz Limited, Surrey, England) (Clark and Smith, 1990). Successful induction of MSD was confirmed by pairing male rats (in ratio 1:1) with sexually mature and experienced female rats which had previously been primed by sequential subcutaneous administration of 10 µg/100 g BW of oestradiol conjugate (Lot No. L95455, Pfizer limited, Kent, United Kingdom) (48 h before pairing) and intramuscular administration of 0.5 mg/100 g BW of progesterone (Lot No. 170811, Zhejiang Jinling Tianfeng Pharmaceutical, Huzhou City, Zhejiang, China) (4 h before pairing) (Amin *et al*., 1996). Oestrous phase in female rats was confirmed by vaginal smears examinations according to OECD-106 guideline (OECD, 2009). The rats were paired for 30 minutes and a video recording was made. The video was later played back to and the following monitored sexual behaviour parameters were counted: Mount Frequency [The number of mounts without intromission from the time of introduction of the female until ejaculation], Intromission Frequency [The number of intromissions from the time of introduction of the female until ejaculation], Ejaculation Frequency [The number of ejaculations throughout the observation period.], Mount Latency [The time interval between the introduction of the female to the first mount by the male], Intromission Latency [The interval from the time of introduction of the female to the first intromission by the male], Ejaculation Latency [The time interval between the first intromission and ejaculation. This is characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity] and Post Ejaculatory Interval [The time interval between ejaculation and erection of the male copulatory organ for the next phase of sexual cycle] (Amin *et al*., 1996; Agmo, 1997). Sexual dysfunction was considered successfully induced in male rats which showed minimum of 25 % reduction in MF, IF and EF as well as minimum increase of 25 % in ML, IL, EL and PEI (Malviya *et al*., 2011).

Animal grouping and extract administration
To evaluate the effect of AREGE on biochemical parameters of MSF, thirty male rats, 25 of which MSD has been successfully induced, were used. Five (5) rats represented sham control (Group A) and received distilled water only. The 25 SD rats were randomly assigned into 5 groups (i. e. B, C, D, E and F) with each group having 5 rats, and administered distilled water, Kongy (reference standard [13.43 mg/kg BW]) and AREGE at 50, 100 and 200 mg/kg BW respectively. Rats were treated for 7 days and sacrificed 24 h after the last extract administration. Their blood and testes were collected, prepared appropriately and used immediately for determination of selected associated biochemical parameters of MSF.
For the toxicity evaluation, 40 male rats were grouped into W, X, Y and Z, each group containing 10 rats, and administered distilled water, 50, 100 and 200 mg/kg BW of AREGE respectively. Five rats per group were sacrificed 24 h after treatment for 1 d while the rest were sacrificed 24 h after treatment for 21 d. Their blood, livers and kidneys were collected and prepared for analysis of liver and kidney function indices.

Preparation of serum and organ homogenization
Blood samples collected in sterile vacutainer bottles were left to clot and thereafter centrifuged (Allegra X-30, Beckman Coulter Life Sciences, Indianapolis, USA) at 9000 rpm for 5 min. Serum samples obtained were then collected into plain tubes using Pasteur's pipette. Testes, livers and kidneys excised from the rats were immediately stored in sterile bottles containing ice-cold 0.25 M sucrose solution and thereafter homogenized (Mixer homogenizer 115V, Thomas Scientific, Swedesboro, NJ 08085, USA) in ice-cold 0.25 M sucrose in an ice bath. Homogenates were then centrifuged at 7000 rpm for 5 min at 4 ^{o}C, and supernatants collected into sample containers for determination of selected biochemical parameters.

Determination of biochemical parameters
Biochemical assays were done using SpectraMax 340PC384 Absorbance Microplate Reader, Molecular Devices, San Jose, California, USA. The methods described by Tietz (1995) were used for the determination of testosterone, AST, ALT, total protein, creatinine, phosphate and calcium. LH was determined by the method of Cumming *et al.* (1985) while NO was determined by the method of Joharchi and Jorjani (2007). The method described by Sartorius *et al*. (2014) was employed for the assay of DHT while that described by Vanderpump *et al*. (1998) was used to determine prolactin. Sodium, potassium, ALP were determined as described by Trinder (1951), Henry (2001) and Wright *et al*. (1972) respectively. GGT, urea and albumin were respectively determined by the methods of Szasz (1976), Fawcett and Scott (1960) and Tietz (1976).

Statistical analysis
Laboratory-generated data were subjected to statistical analysis using the IBM® Statistical Package for Social Sciences (SPSS) software version 20. All significant differences were determined by one way Analysis of Variance (ANOVA) and Post Hoc multiple comparisons was done using Duncan's multiple range test. The significance level was set at $p < 0.05$. Results are presented as mean (of 5 determinations) ± standard error of mean (SEM).

RESULTS

Secondary metabolites composition of aqueous root extract of Gardenia erubescens
Alkaloids, tannins, flavonoids and steroids were detected in AREGE with flavonoids having the highest concentration. Terpenoids, anthraquinones and cardiac glycosides were not detected (Table 1).

Mineral constituents of aqueous root extract of Gardenia erubescens

A total of fourteen minerals were detected, which included iron, molybdenum, copper, zinc and selenium amongst others. Iron (Fe) had the highest concentration (456.00 ppm) while Cobalt (Co) was the lowest (1.00 ppm) (Table 2).

Amino acid composition of aqueous root extract of Gardenia erubescens

Eighteen of the twenty standard amino acids were detected in AREGE. Glutamic acid had the highest concentration (6.81 g/100g) while tryptophan was lowest at 0.70 g/100g. Glutamate and aspartate were not detectable.

Biochemical parameters of male sexual function

Clonidine administration to male rats caused a significant reduction ($p < 0.05$) in MF (47.06%), IF (30.96%) and EF (26.75%) while the latencies of mount, intromission and ejaculation and post ejaculatory interval were prolonged by 30.14%, 30.28%, 51.32% and 32.63% respectively when compared with distilled water-treated control group (Table 4).

Administration of clonidine significantly reduced ($p < 0.05$) serum and testicular testosterone, serum LH, DHT and prolactin levels in the rats when compared with the distilled water-treated control group whereas penile NO level was not altered ($p > 0.05$) in the rats when compared with the distilled water-treated control group (Figure 1 and 5).

The extract at 200 mg/kg BW caused serum testosterone level of the rats to increase significantly ($p < 0.05$) to a level comparable with the distilled water-treated control (Figure 1). All the doses of AREGE significantly increased ($p < 0.05$) testicular testosterone concentration, with the 50 and 100 mg/kg BW doses comparing favourably with the distilled water-treated control while the 200 mg/kg BW dose increased testicular testosterone to a level higher ($p < 0.05$) than the distilled water-treated control but that compares with the Kongy-treated group (Figure 1).

Similarly, AREGE (100 and 200 mg/kg BW) increased serum LH level significantly ($p < 0.05$) when compared with the clonidine and distilled water-treated rats. Only the 200 mg/kg BW-treated group compared favourably with the distilled water-treated control group (Figure 1).

Administration of AREGE significantly increased ($p < 0.05$) the levels of serum DHT when compared with the clonidine and distilled water-treated rats, however only the rats administered 200 mg/kg BW of AREGE compared favourably with the distilled water-treated control and Kongy-treated rats. Serum prolactin level was also raised significantly by the extract (50 and 100 mg/kg BW) to a level comparable with the distilled water-treated control and the Kongy-treated rats. Similarly, AREGE at all doses elevated penile level of NO when compared to the clonidine and distilled water-treated rats. The recorded increase was significantly higher ($p < 0.05$) than the distilled water-treated control and the Kongy-treated group.

Kidney and liver function indices

Administration of AREGE to rats did not alter kidney and serum activities of ALP on day 1, however, there was a significant reduction ($p < 0.05$) in kidney ALP activity after AREGE (100 and 200 mg/kg BW) administration for 21 days (Figure 3).
On day 1 of AREGE administration, GGT activity in the kidney was significantly reduced ($p < 0.05$) by AREGE at all doses when compared with the control, while in the serum, GGT activity was significantly increased ($p < 0.05$) by all the doses of AREGE when compared with the control. On day 21, the 100 and 200 mg/kg BW of AREGE caused a significant decrease ($p < 0.05$) in kidney GGT activity and an increase in serum GGT activity when compared with the control (Figure 3).

Serum concentrations of potassium and calcium ions were not altered by AREGE at all doses on days 1 and 21 (Figure 4). There was however a significant increase ($p < 0.05$) in sodium ion concentration at day 1 of AREGE administration (all doses) when compared with the control. Similarly, phosphate ion concentration was also increased at day 1 by AREGE at 200 mg/kg BW. However, at day 21 of treatment, there was no significant difference ($p > 0.05$) in serum concentrations of sodium and phosphate ions of AREGE-treated rats when compared with the control (Figure 4). Serum creatinine concentration was significantly elevated ($p < 0.05$) in the rats treated with AREGE (at 100 and 200 mg/kg BW) for 1 and 21 days when compared with the control. The concentration of urea in the serum was also significantly raised ($p < 0.05$) in the rats treated with AREGE at all doses for 1 day when compared with the control. There was no significant difference ($p > 0.05$) in urea concentration in the AREGE-treated rats when compared with control after 21 days (Figure 5).

At day 1 administration of AREGE significantly increased ($p < 0.05$) liver (50 and 200 mg/kg BW) and serum (50 mg/kg BW) AST activity when compared with the control. In the serum, AST activity was similarly significantly increased ($p < 0.05$) by AREGE at 50 mg/kg BW (Figure 6). At day 21, AREGE (100 mg/kg BW) significantly increased ($p < 0.05$) liver AST activity when compared with the control while in the serum, AREGE (at all doses) caused a significant rise ($p < 0.05$) in AST activity when compared with the control.

There was no alteration of liver and serum ALT activity by AREGE at day 1. Meanwhile at day 21, AREGE (at all doses) significantly increased ($p < 0.05$) liver ALT activity when compared with control but there was no alteration ($p > 0.05$) in serum ALT activity when compared with control (Figure 6).

In AREGE-treated rats, there was no significant difference ($p > 0.05$) in serum concentrations of albumin (days 1 and 21) and total protein (day 21) when compared with the control. Total protein concentration was significantly reduced ($p < 0.05$) in rats treated with AREGE (50 mg/kg BW) (Figure 7).

Table 1: Secondary metabolites composition of aqueous root crude extract of *Gardenia erubescens*

Secondary Metabolites	Concentration (%/2g sample)
Alkaloids	7.70 ±0.32
Tannins	6.50 ±0.15
Flavonoids	12.20 ±1.22
Steroids	2.00 ±0.26
Terpenoids	ND
Anthraquinones	ND
Cardiac glycosides	ND

Values are means (of 2 replicates) ± SD
Key:
ND = Not detected

Table 2: Mineral composition of aqueous root crude extract of *Gardenia erubescens*

Mineral	Amount (ppm)
Iron (Fe)	456.00 ±5.32
Strontium (Sr)	38.00 ±1.52
Niobium (Nb)	9.00 ±0.67
Molybdenum (Mo)	9.00 ±1.55
Cobalt (Co)	1.00 ±0.02
Nickel (Ni)	12.00 ±1.32
Copper (Cu)	18.00 ±1.04
Zinc (Zn)	18.00 ±0.32
Selenium (Se)	3.00 ±0.12
Rubidium (Rb)	27.00 ±1.34
Zirconium (Zr)	6.00 ±0.45
Mercury (Hg)	5.00 ±0.33
Lead (Pb)	5.00 ±0.58
Uranium (U)	2.00 ±0.11

Values are means (of 2 replicates) ± SD

Table 3: Amino acid composition of aqueous root crude extract of *Gardenia erubescens*

Amino Acid	Concentration (g/100g)
Leucine	4.90 ±0.11
Lysine	3.70 ±0.04
Isoleucine	3.01 ±0.45
Phenylalanine	2.66 ±0.52
Tryptophan	0.70 ±0.02
Valine	3.40 ±0.18
Methionine	1.01 ±0.03
Proline	3.04 ±0.02
Arginine	3.35 ±0.12
Tyrosine	2.41 ±0.13
Histidine	2.01 ±0.12
Cystine	0.85 ±0.03
Alanine	3.00 ±0.02
Glutamic acid	6.81 ±0.52
Glycine	3.11 ±0.15
Threonine	2.33 ±0.06
Serine	3.40 ±0.18
Aspartic acid	4.60 ±0.54

Values are means (of 2 replicates) ± SD

Table 4: Sexual behavior parameters in male rats following oral administration of clonidine

Parameters	Distilled Water (control)	Clonidine (0.5 mg/kg BW)	Change (%)
Mount Frequency	0.85±0.01	0.45±0.01*	47.06↓
Intromission Frequency	3.65±0.44	2.52±0.04*	30.96↓
Ejaculation Frequency	15.70±1.70	11.50±0.03*	26.75↓
Mount Latency	19.54±0.37	25.43±0.21*	30.14↑
Intromission Latency	19.95 ± 0.21	25.99±0.26*	30.28↑
Ejaculation Latency	0.76±0.08	1.15±0.07*	51.32↑
Post-ejaculatory Interval	13.67 ± 1.34	18.13±0.43*	32.63↑

Data are mean ± SD; n= 5; *p < 0.05, versus control; ↓= Percentage decrease, ↑= Percentage increase

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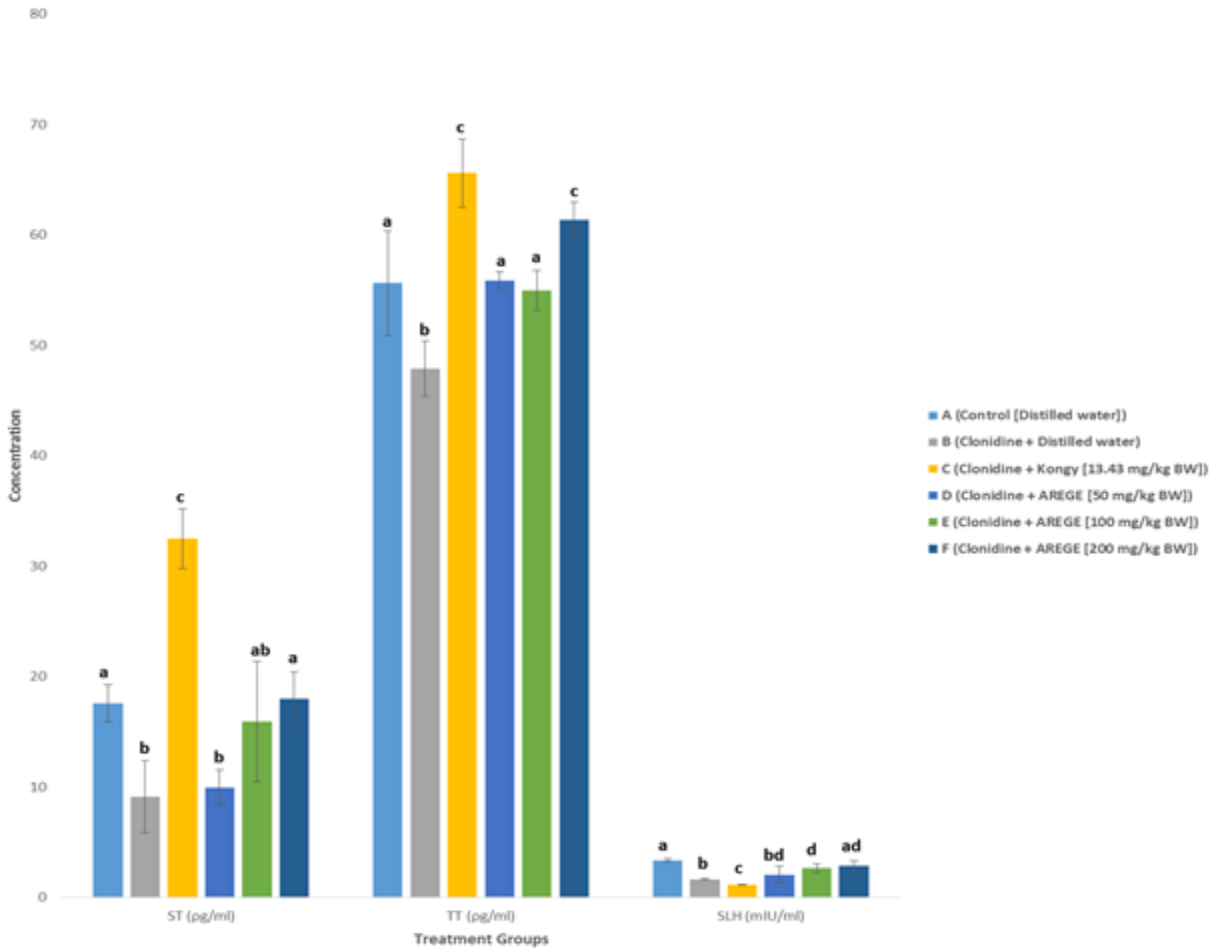

Figure 1: Serum and testicular testosterone and serum luteinizing hormone concentrations in clonidine-induced sexually dysfunctional rats administered aqueous extract of *Gardenia erubescens*

Data are expressed as means ± SD; n = 5; Bars carrying different superscripts are significantly different at $p < 0.05$; AREGE: Aqueous root extract of *Gardenia erubescens*.
ST: Serum testosterone
TT: Testicular testosterone
SLH: Serum luteinizing hormone

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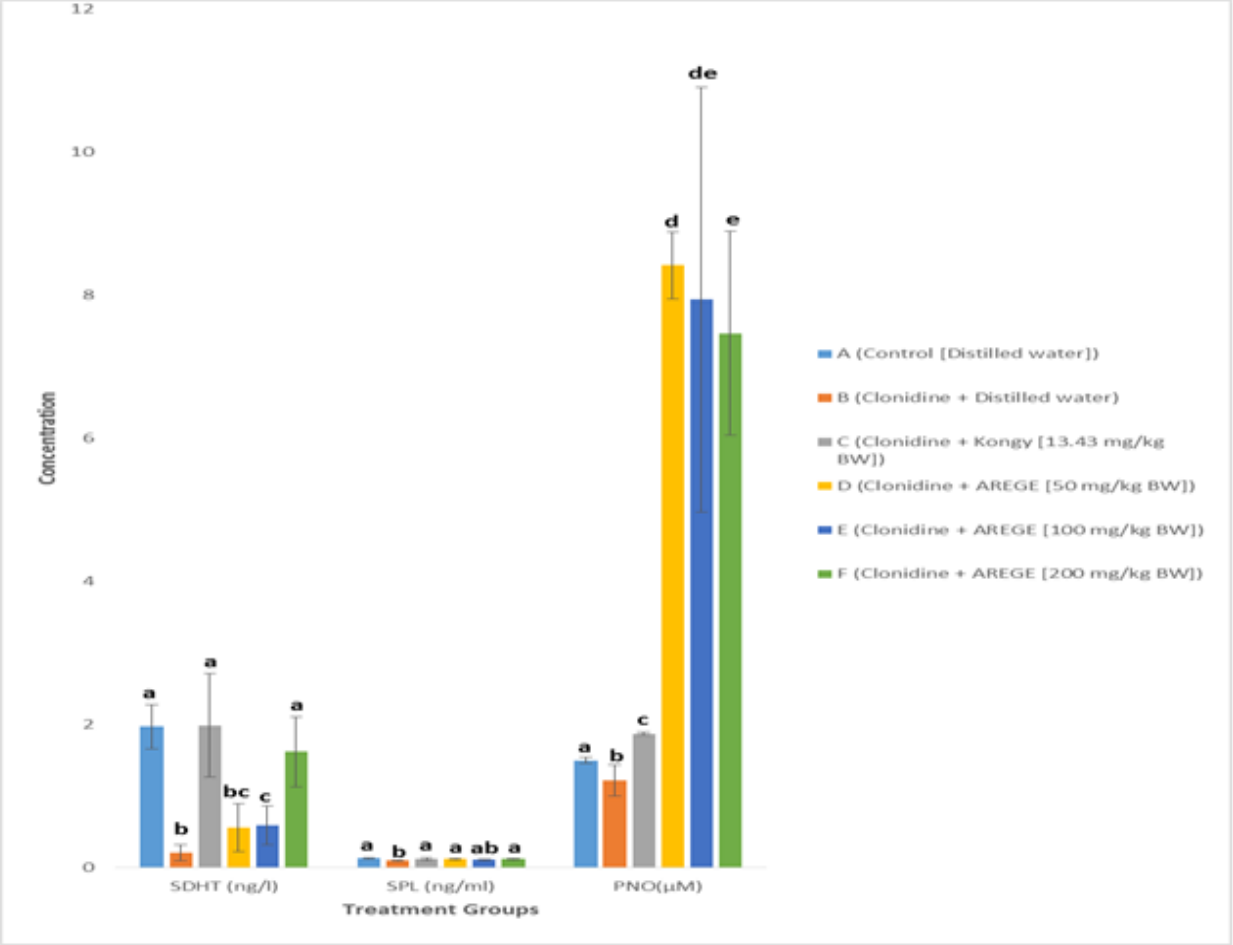

Figure 2: Serum dihydrotestosterone, serum prolactin and penile nitric oxide concentrations in clonidine-induced sexually dysfunctional rats administered aqueous extract of *Gardenia erubescens*

Data are expressed as means ± SD; n = 5; Bars carrying different superscripts are significantly different at $p < 0.05$; AREGE: Aqueous root extract of *Gardenia erubescens*.
SDHT: Serum dihydrotestosterone
SPL: Serum prolactin
PNO: Penile nitric oxide

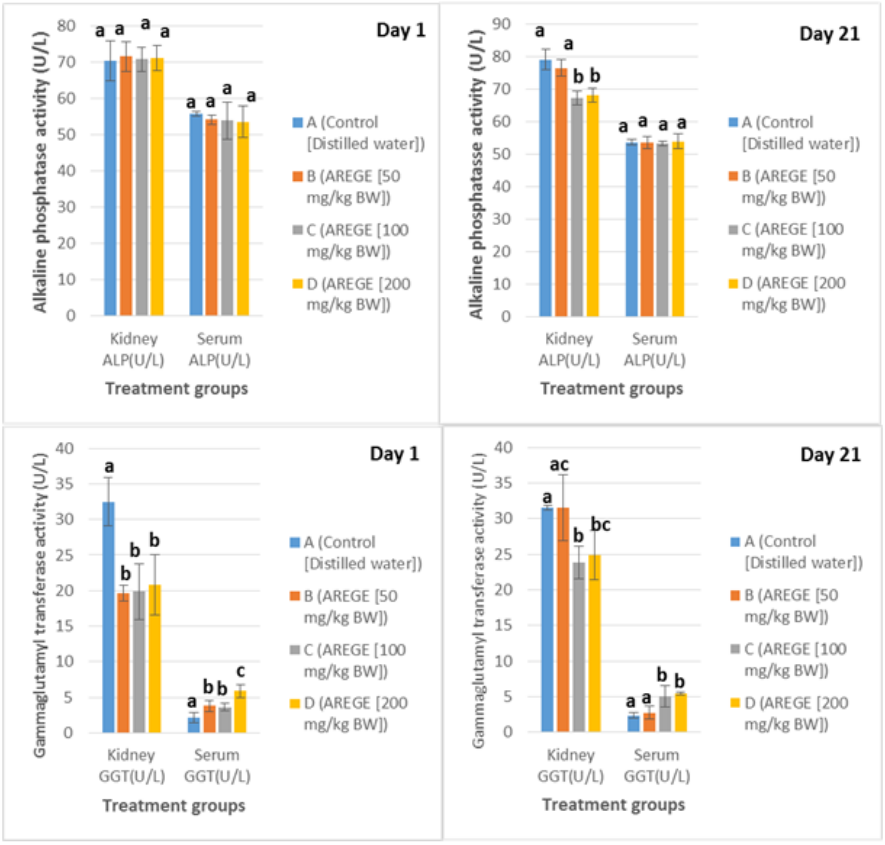

Figure 3: Kidney and serum alkaline phosphatase and gammaglutamyl transferase activities in rats treated with aqueous root extracts of *Gardenia erubescens*

Data are expressed as means ± SD; n = 5; Bars carrying different superscripts are significantly different at $p < 0.05$; AREGE: Aqueous root extract of *Gardenia erubescens*.
ALP: Alkaline phosphatase
GGT: Gammaglutamyl transferase

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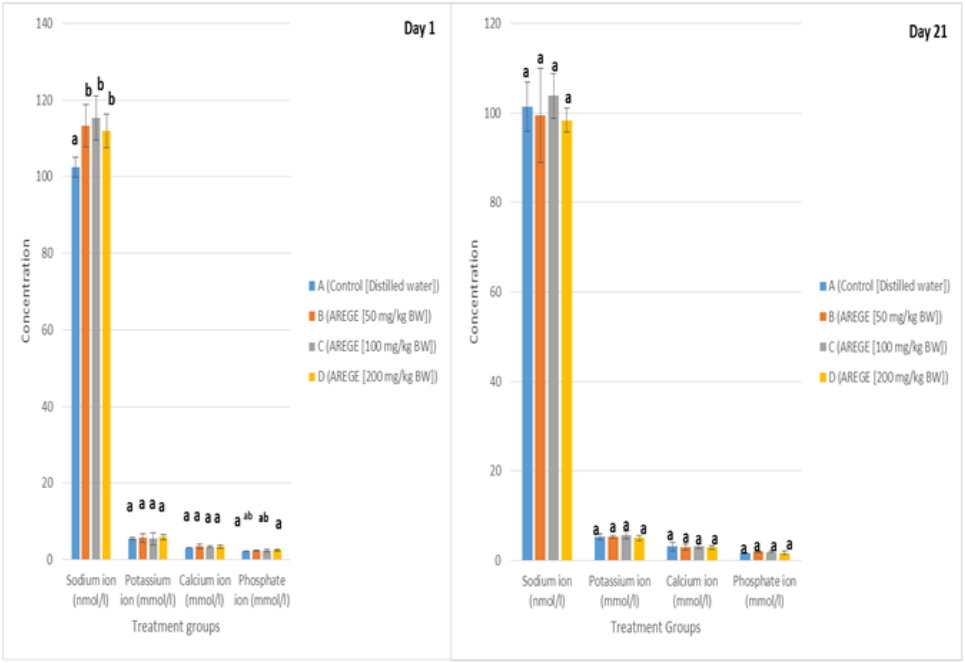

Figure 4: Selected serum electrolyte concentrations in rats treated with aqueous root extracts of *Gardenia erubescens*

Data are expressed as means ± SD; n = 5; Bars carrying different superscripts are significantly different at $p < 0.05$;
AREGE: Aqueous root extract of *Gardenia erubescens*.

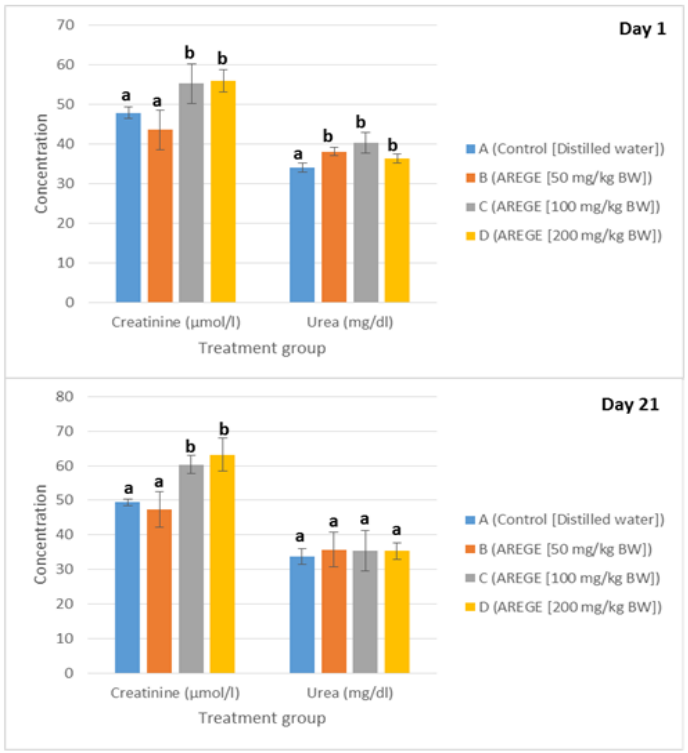

Figure 5: Serum creatinine and urea concentrations in rats treated with aqueous root extract of *Gardenia erubescens*

Data are expressed as means ± SD; n = 5; Bars carrying different superscripts are significantly different at $p < 0.05$;
AREGE: Aqueous root extract of *Gardenia erubescens*.

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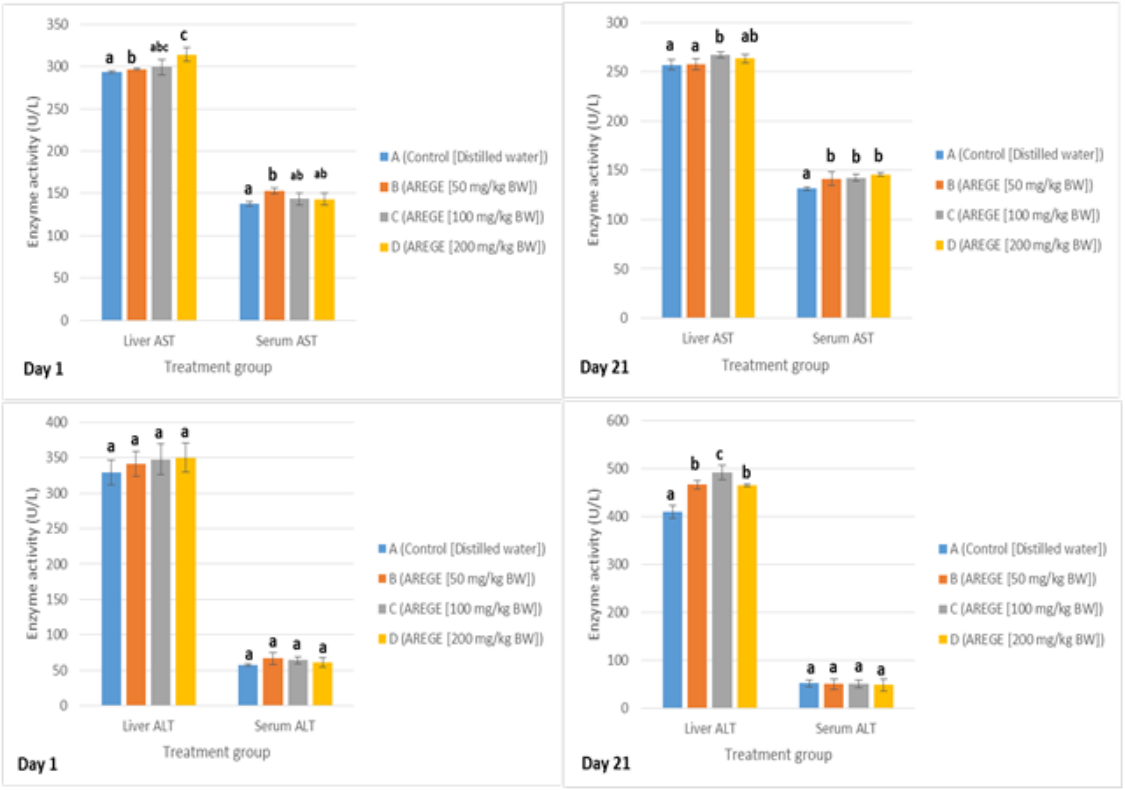

Figure 6: Liver and serum aspartate aminotransferase and alanine aminotransferase activities in rats treated with aqueous root extracts of *Gardenia erubescens*

Data are expressed as means ± SD; n = 5; Bars carrying different superscripts are significantly different at $p < 0.05$;
AREGE: Aqueous root extract of *Gardenia erubescens*.
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase

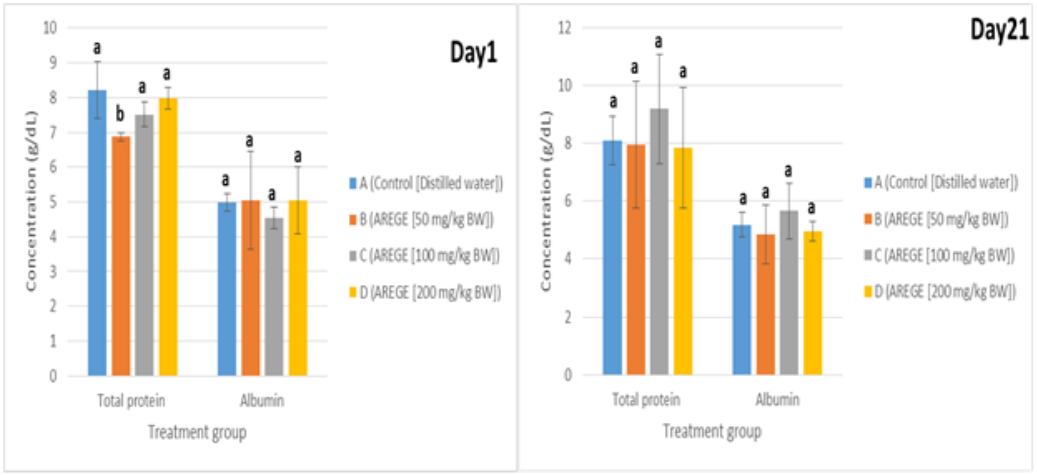

Figure 7: Acute and sub-acute serum concentrations of total protein and albumin in rats treated with aqueous root extract of *Gardenia erubescens*

Data are expressed as means ± SD; n = 5; Bars carrying different superscripts are significantly different at $p < 0.05$;
AREGE: Aqueous root extract of *Gardenia erubescens*

DISCUSSION

Plant secondary metabolites have been reported to enhance male sexual functions. Saponins boost LH and FSH levels leading to increased testosterone and consequently enhance sexual behaviour. In this study, the recorded increase in LH and testosterone could be attributed to saponins present in AREGE. Saponins might have acted by inducing the production of LH, which in turn might have either increased the levels of precursors necessary for the synthesis of testosterone and/or suppressed enzymes that convert testosterone into other metabolites (Golan *et al*., 2008; Stadtlander *et al*., 2012). Saponins also influence the NO signaling pathway by enhancing the activity of NO synthase (NOS) which in turn increases penile NO content, causing increased blood flow to the penis and finally culminating in penile erection (Chauhan *et al*., 2014). Kim *et al*. (1998) reported that saponins of *Panax ginseng* induced relaxation of the corpus cavernosum muscle by acting as NO donor through the L-arginine/NO pathway. Wang *et al*. (2010) also submitted that a saponin, ginsenoside from ginseng, induced the synthesis of NO, increased serum testosterone concentration and facilitated copulatory behaviour in male rats. Alkaloids and flavonoids promote vascular relaxation by stimulating NO production, protecting the synthesized NO against reactive oxygen species and inhibiting phosphodiesterase V (Yakubu *et al*., 2011; Yakubu and Atoyebi, 2018; Rouamba *et al*., 2018). Also, flavonoids alter androgen levels (thus enhancing sexual stimulation), increase superoxide dismutase and catalase activities, thereby imparting an indirect potentiating effect on the sexual behaviour parameters (Yakubu *et al*., 2011). The secondary metabolites profile of the aqueous extract of *G. erubescens* root in this study is essentially similar to that of the plant bark reported earlier by Ayeni and Kayode (2019) in their paper on ethnobotanical survey of plants' stem barks used in Kaduna State of Nigeria.

Iron (Fe), Copper (Cu), Zinc (Zn) and Selenium (Se) have been implicated to play important roles in the sexual functioning of animals. The mineral profile of AREGE in this study is similar to that of the fruit of *G. erubescens* reported earlier by Rouamba *et al*. (2018). There is an association between serum testosterone levels and body iron indices in humans (Gabrielsen, 2017; Karunanithi *et al*., 2019). Iron is involved in peroxidase and catalase synthesis, which are enzymes involved in antioxidation. Antioxidation functions to prevent diseases and stress, which enhances male sexual behavior (Kayode and Yakubu, 2017). Zinc is an aphrodisiac and is important in the production of testosterone and reduction of oxidative stress in developing sperm cells (Kayode and Yakubu, 2017; Miska-Schramm *et al*., 2018). Like zinc, copper is positively associated with testosterone, and a positive relation between the levels of testosterone and LH has also been described (Hallinan, 2011). Selenium keeps the testes and seminal vesicles healthy and promotes the production of sperm and its motility. It plays a vital role in modulation of antioxidant defense mechanisms and other essential biological pathways and redox sensitive transcription factors (Braverman, 2008; Qazi *et al*., 2019). Therefore, the presence of these minerals, among others in AREGE (Table 2) either acting singly or synergistically may confer the ameliorative and restorative effects of AREGE on the Clonidine-treated male rats.

Glutamine, arginine, tryptophan, tyrosine and phenylalanine which have been implicated to play vital roles in normal sexual functioning of animals were present in AREGE. These findings correlate with previous research by Kayode and Yakubu (2017). Orgasm is regulated by the amount of gamma-aminobutyric acid (GABA), obtained from glutamine and inositol. Gamma-aminobutyric acid inhibits excitatory neurotransmitters that cause anxiety related loss of sexual interest and consequently SD (Jung *et al*., 1997). In addition, NO from arginine increases arterial elasticity, reduces blood pressure and improves erection and sexual performance (Stanislavov and Nikolova, 2003; Palloshi *et al*., 2004). Restoration of 80% of sexual ability in men treated with combination of L-arginine and pycnogenol for two months have been reported by Stanislavov and Nikolova (2003), suggesting a role for L-arginine in enhancing sexual activity in men. Tryptophan may also play a role in the synthesis of serotonin, via tryptophan hydroxylase, whereas phenylalanine can undergo hydroxylation, catalyzed by phenyalanine hydroxylase to give L-tyrosine and subsequently L-3,4-dihydroxyphenylalanine, dopamine, norepinephrine, and epinephrine (adrenaline), making these chemical compounds candidates for enhancing libido. Glycine, cysteine and glutamic acid in AREGE could serve as precursors of glutathione, an antioxidant that protects cells against the pathogenesis of many diseases including those of sexual origin (Imaga *et al*., 2010).

Induction of sexual incompetence in rodents by clonidine has been previously reported (Clark and Smith, 1990; Srilatha *et al*., 1999; Lin *et al*., 2015). Clonidine may induce SD inhibition of sympathetic outflow from the brainstem, which directly influences erection, reduction of noradrenergic stimulation of postsynaptic α-adrenoceptors, which can affect ejaculation, depletion of central neurotransmitters which leads to decreased libido and/or through its sedative and depressant properties (Forman *et al*., 2005).

Clonidine reduced MF, IF and EF and prolonged ML, IL, EL and PEI by more than the 25 % bench mark stipulated by Malviya *et al*. (2011). A reduction in MF implies reduced sexual motivation, while decreased IF indicates reduced ability to obtain and sustain erection coupled with bad penile orientation that will make vaginal penetration difficult. A low EF connotes a poorly activated ejaculatory reflex (Abedi *et al*., 2013). On the other hand, prolonged ML and IL indicate impaired sexual motivation and stimulation, prolonged EL suggests prolonged duration of coitus beyond normal while prolonged PEI implies reduced sexual potency/desire, exhaustion and poor recovery after the first round of sexual activity which will translate to a reduced intensity of sexual behavior in next rounds of mating (Fouche *et al*., 2015). The clonidine-associated reduction in testosterone, DHT and NO corroborate the result obtained for the sexual behavior parameters. The clonidine-related reduction in the biochemical parameters associated with MSF was reversed by AREGE, especially at the highest dose (200 mg/kg BW). The effectiveness of the plants used as aphrodisiac is believed to be through various mechanisms such as vasodilation, elevation of androgens and gonadotropin, maintaining the level of prolactin and generation of NO (Buntin and Tesch, 1985; Fouche *et al*., 2015; Anawalt, 2017).

Testosterone acts on androgen receptors to stimulate the components of sexual behavior which include sexual desire, motivation, arousability, sexual fantasies, penile rigidity and sexual performance (Fouche *et al*., 2015; Kayode and Yakubu, 2017; Yakubu and Atoyebi, 2018). In this study, clonidine-related decrease in the serum and testicular testosterone were not only

reversed, but testosterone was actually increased by AREGE, especially at 200 mg/kg BW. The effect of AREGE on testosterone was dose-dependent. The ability of AREGE to induce testosterone production could be considered as one of the contributing factors responsible for its use as a male sexual performance enhancer. Yakubu and Atoyebi (2018) had previously reported similar increase in the level of testosterone after the administration of *Brysocarpus coccineus* root to male rats. This inductive effect of AREGE on testosterone is similar to those obtained with the reference drug, Kongy. The 200 mg/kg BW of AREGE showed the best results for both serum and testicular testosterone.

LH is produced by the anterior pituitary lobe in response to declining testosterone levels, but an increase in testosterone normally induce reduction in LH level through feedback inhibition. The extract reversed clonidine-mediated reduction in LH level. It is also possible that the extract or its components acted on the hypothalamic-anterior pituitary-gonadal axis to bring about an increase in both the synthesis and secretion of testosterone and LH.

Nitric oxide is the principal mediator of penile erection (Lasker *et al*., 2013). Increase in NO level enhance the relaxation of smooth muscles, stimulate blood flow into the erectile bodies of the corpus cavernosum and consequently promote penile erection (Hull *et al*., 1994; Lasker *et al*., 2013). Clonidine may inhibit NO synthase (NOS) activity, thus reducing NO production (Giorgio *et al*., 2000). In this study, the recorded elevation of NO by all doses of AREGE implies increased biosynthesis of NO occasioned by extract administration. The extract, acting as a reservoir of arginine (starting material for NO biosynthesis), might have released the amino acid to the tissue/cellular system of the rats for the enhanced overall synthesis of NO (Salas-Huetos *et al*., 2019). Consequently, an increase in either NOS or NO through the accumulation of cyclic guanosine monophosphate (cGMP), can cause the blood vessels of the penile tissue to become engorged with blood and promote penile erection (Corbin, 2004).

Dihydrotestosterone (DHT) is an endogenous androgen sex steroid and hormone. Relative to testosterone, DHT is considerably more potent as an agonist of the androgen receptor (AR). Testosterone is converted to DHT with the help of enzyme 5α-reductase (Karunanithi *et al*., 2019). Suppression of circulating and tissue DHT concentrations is associated with decreases in libido, erectile function and average sperm concentration (Anawalt, 2017). In this study, the highest dose (200 mg/kg) of the extract was able to restore the DHT level back to normal levels. Extract may contain molecules which are capable of increasing either the concentration or activity of 5α-reductase, thus facilitating testosterone conversion to DHT. It is also possible that extract inhibited an enzyme like aromatase, which catalyses the conversion of testosterone to oestradiol, thus ensuring that testosterone is available for conversion to DHT.

Prolactin is polypeptide hormone that is synthesized and secreted from specialized cells of the anterior pituitary gland, the lactotrophs (Freeman *et al*., 2000). Prolactin (PRL) plays diverse roles in men's reproduction and health (Bolyakov and Paduch, 2011). Hyperprolactinemia may present as infertility, decreased sex drive, ED, decreased volume of ejaculate and anorgasmia in men (Bolyakov and Paduch, 2011). Hyperprolactinemia also impairs the pulsatile LH release, which results in a decrease of serum testosterone secretion (Buvat, 2003). While an increase in prolactin impacts negatively on male sexual functions, a physiological concentration of circulating prolactin seems to be involved in the central control of sexual behavior and activity, by modulating mainly the effects of dopaminergic and serotoninergic systems on sexual functions (Galdiero *et al*., 2012). In this study, clonidine-induced drop in serum prolactin levels was restored to normal levels by the extract where its maximum sexual function effect is obtainable.

In the toxicity study, there was no mortality or any toxic manifestations observed at any of the doses selected throughout the study period. The biochemical indices of liver and kidney damage monitored in this study are useful markers for assessing the functional capacities of the organs (Appidi *et al*., 2009).
Alkaline phosphatase (ALP) is a 'marker' enzyme of damage for the plasma membrane and endoplasmic reticulum (Ashafa *et al*., 2009). The recorded decrease in serum ALP in the groups that received 100 and 200 mg/kg BW of extract for 21 days suggests either inhibition of the enzyme activity at the cellular/molecular level, inactivation of the enzyme molecules *in situ* and/or repression of enzyme synthesis (Yakubu and Nurudeen, 2014).
Gammaglutamyl transferase (GGT), a membrane bound microsomal enzyme present in the hepatocytes, renal tubules, pancreas, and intestine is also located within the cell membrane where they transport peptides into the cell and across the cell membrane (Ashafa *et al*., 2009, Ajiboye *et al*., 2010). The recorded depletion in kidney GGT level which instantly reflected as a concomitant elevation in the serum implies a compromise of plasma membrane integrity resulting to leakage of the enzyme into the serum. However, it is also possible that extract repressed/inhibited GGT synthesis/activity as recorded with ALP, so that the recorded increase in serum GGT may have originated from other predominant sources such as liver, heart, pancreas, and brain. The latter is more likely the case since a derangement of plasma membrane of kidney cells would have permitted the leakage of both ALP and GGT, which are both membrane enzymes.

One of the functions of the kidneys is the maintenance of electrolyte homeostasis (Pollock *et al*., 2014). Alteration in serum concentrations of electrolytes such as Na^+, Ca^{2+}, K^+ and PO_4^{3-} may indicate renal dysfunction at both the tubular and glomerular levels (Yakubu and Musa, 2012). The recorded increase in serum sodium ion after administration of AREGE for 1 day is an indication of decreased reabsorption suggestive of renal tubular dysfunction which may result in hypernatremia, a disorder of sodium homeostasis resulting from increased serum level of sodium (Ackerman *et al*., 1990). The lack of effect on potassium and calcium ions at all doses of AREGE used and the elevation of the serum levels of sodium (all doses) and phosphate (200 mg/kg BW) ions suggests that the extract may have selective toxicity. However, the rats recovered from the assault posed by the extract at day 21.

Serum urea and creatinine are indices used to assess kidney functions (Soji-Omoniwa *et al*., 2014). Creatinine is formed by non-enzymatic breakdown of creatinine phosphate and an elevation in its level in the serum indicates impaired glomerular filtration and clearly suggests renal failure (Yakubu and Musa, 2012; Soji-

Omoniwa *et al*., 2014). In this study, the recorded increase in the level of serum creatinine after the administration of AREGE for 1 day (at 100 and 200 mg/kg doses) may have resulted from increased physical activity in the rats, leading to increased creatine catabolism (Alessandra *et al*., 2008). Urea is a major non-protein nitrogenous catabolite of protein metabolism (Gowda *et al*., 2010; Salazar, 2014). The body's dependency on the renal system to excrete urea makes it a useful analyte to evaluate renal function (Salazar, 2014). High blood urea levels in renal disease are a consequence, not a cause, of impaired renal function (Soji-Omoniwa *et al*., 2014). The recorded elevation of serum urea level following administration of AREGE for 1 day may be attributed to increased protein catabolism, which occurred at a rate that far exceeds the rate of its excretion by the kidneys. Rats recovered from the shock occasioned by AREGE administration after 21 days of treatment.

Aspartate aminotransferase (AST) and ALT are two of the most reliable markers of hepatocellular injury or necrosis (Omoniwa *et al*., 2021). Although AST is less specific than ALT as a marker of liver damage, elevation in the serum levels of the two enzymes is an indicator of tissue damage and altered membrane permeability (Kolawole *et al*., 2013). The recorded increase in the level of liver AST and ALT after administration of AREGE may be due to induction of the enzyme synthesis or activation of their activities. Overproduction or increase in the activities of AST and ALT will directly cause an increase in protein degradation and may be the reason for the recorded upsurge in serum level of urea in the extract-treated rats. The concomitant elevation of serum levels of AST and ALT may either be attributed to leakage from the liver or other principal sources not considered in this study. It is possible that the rate of induction of these enzymes in liver cells far exceeds the rate of leakage into the extracellular space, which may explain the recorded simultaneous elevation of kidney and serum AST and ALT levels.

Serum total protein and albumin are synthesized in the liver and are thus used as indices for measuring the synthetic function of the liver (Omoniwa *et al*., 2014). Total protein defines the balance of protein synthesis and catabolism. Increased serum protein occurs in cases of liver cirrhosis (Salau *et al*., 2019). The recorded decrease in serum total protein level at day 1 of AREGE administration might imply that the extract hindered protein production thus compromising liver synthetic function. Since albumin was unaltered by AREGE administration, it is safe to conclude that the extract selectively inhibited globulin synthesis.
These potential adverse effects of this extract may be attributable to the presence of alkaloids, tannins and saponins in the extracts. Although mechanism of action of these secondary metabolites as it relates to toxicity was not evaluated in the present study, some of these metabolites have been found to exert toxic effects to the liver and kidney (Salau *et al*., 2019).

This research was conducted using personally-generated funds. Access to more funds would have given us the opportunity to increase the number of replicates used in this work which would have helped reduce errors. Also more funds would have enabled us to conduct other experiments like evaluation of extract effects on histology of the tissues studied and effects on physical and behavioural parameters of MSF in the clonidine-treated rats.

Conclusion

Findings of this study suggest that AREGE, especially at 200 mg/kg, possesses the ability to increase the selected biochemical parameters of MSF. These findings justify the use of the root decoction of *G. erubescens* in folk medicine as a MSF enhancer, and imply that it could be a possible candidate for developing drugs for managing MSD. However, AREGE exhibited hepatotoxic and nephrotoxic activities in the rats. Therefore, the plant is not completely safe and should be used with caution.

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