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Full Length Research Article

TOXICITY AND MODULATING EFFECT *OF OLAX SUBSCORPIODEA* OLIV. AQUEOUS ROOT EXTRACT ON CLONIDINE-INDUCED SEXUAL DYSFUNCTION IN MALE WISTAR RATS

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ABSTRACT
This study examined the effects of aqueous root extract of *O. subscorpioidea* Oliv. (AREOS) on liver function, male sexual hormones and nitric oxide in normal and clonidine-treated Wistar rats. Thirty rats were divided into six groups to assess sexual function parameters, including a control group, a clonidine hydrochloride-only group, and groups receiving clonidine plus Adam's desire or varying doses of AREOS (250, 500, 1000 mg/kg body weight). After seven days of treatment, blood and testicular samples were analyzed for testosterone (TT), serum testosterone (ST), luteinizing hormone (LH), follicle-stimulating hormone (FSH), dihydrotestosterone (DHT), and nitric oxide (NO). Toxicity study was also conducted on 20 male rats treated with different doses of AREOS to analyze liver enzymes and serum proteins. Phytochemical analysis revealed bioactive compounds like saponins, flavonoids, and amino acids. Clonidine treatment increased TT, ST, and DHT but decreased LH and FSH. AREOS reduced TT, ST, DHT, and NO levels but increased LH, compared to the clonidine only-treated group. AREOS had no significant effects on liver function markers but significantly lowered serum alanine aminotransferase (ALT) activity. Overall, AREOS was not hepatotoxic and showed potential to normalize hormone imbalances, supporting its traditional use in treating male sexual dysfunctions.

Keywords: Clonidine, Nitric Oxide, Transaminase, Testosterone, Liver.

INTRODUCTION

Male sexual dysfunction (MSD) is a series of conditions defined by impaired sexual functioning (Anderson *et al*., 2022). The normal male sexual response cycle have been classified into five interrelated events that occur in a define order: libido, erection, ejaculation, orgasm and detumescence. MSD occurs when there is a challenge with any component of the male sexual response cycle which may present as libido disorder, erectile dysfunction, ejaculation problems, orgasmic disorders, and detumescence failure (Omoniwa *et al*., 2022).

Since MSD is multifaceted, treatment and management options have been devised depending on the specific etiology. However, many of these available options come with shortcomings which makes searching for alternative treatment necessary. For example, penile prosthesis surgery which is an effective treatment for erectile dysfunction, is costly ($8,000-$14,000) and is often avoided due to fears of surgery, anesthesia complications, infection risk and the perceived unnaturalness of inflatable devices (Rodriguez & Pastuszak, 2017), while testosterone replacement therapy (TRT), which is used for treating libido disorders, poses risks, such as exacerbating prostate cancer, worsening benign prostatic hyperplasia, increasing polycythemia, and potentially triggering obstructive sleep apnea (Osterberg *et al*., 2014). In addition, medications like Viagra for erectile dysfunction are generally effective but cause minor side effects like headaches, flushing, visual disturbances, and digestive issues which may deter long-term use (Graziano *et al*., 2017). As a result of these drawbacks, many sufferers, especially those living in developing countries, where access to quality health care is limited, resort to the use of medicinal plants.

O. subscorpioidea Oliv. belongs to the family Olacaceae and is commonly referred to as *Ifon or Ufon (Yoruba), Igbulu*, *Atu-ogili*, Aziza or *Osaja* (Igbo), and *Gwaanon kurmi or Gwaanon raafii (Hausa), Ukpakon (Edo), Ocheja (Igala) and Mtungapwezi (Swahili)* (Victoria *et al*., 2010; Odoma *et al*., 2016). It is widely distributed in West African countries such as Nigeria, Zaire and Senegal (Ayandele & Adebiyi, 2007). *O. subscorpioidea* Oliv. is a shrub or tree which is about 10 meters in height, bole to 60 cm girth with long thin, often drooping branches. The branches are flexible and angular with elliptex leaves; lanceolate in its entire margin. The leaf apex has a characteristic point (mucronate), with about 7 pairs of looped laterals. *O. subscorpioidea* Oliv. fruit is small, globular, about 1.5 cm in diameter, and yellowish but turning red when ripe. It has a slash smell like garlic (Oludare & Adesemoye, 2019). Different body parts of *O. subscorpioidea* Oliv. are employed in different countries for the treatment of various ailments such as orodental infections, inflammatory diseases, convulsion, pain, cancer, diabetes mellitus, obesity, asthma, malaria, constipation, diarrhoea, gastric ulcer, articular pains, venereal diseases, anxiety, mental disorders, infectious diseases, arthritis, rheumatism, hepatic diseases, sexually transmitted diseases, Alzheimer's disease, depression, constipation, yellow fever, jaundice, guinea worm, constipation, cough, dermatosis, fever, headaches, jaundice, malaria, rheumatism, syphilis, ulcer, and many other diseases (Ahmad *et al*., 2021).
Various works on the phytochemistry of the different body parts of *O. subscorpioidea* Oliv. have revealed the presence of saponins, tannins, steroids, cardiac glycosides, flavonoids, alkaloids, terpenoids, phenols, carbohydrates, phlobatannins, athraquinonones, polyphenols, reducing sugars, deoxysugar, cardenolides, triterpenes, calcium, copper, manganese, magnesium, sodium, zinc, potassium, aluminium, silicon,

phosphorus, sulphur, chlorine, iron, cobalt, nickel, bromine, rubidium, strontium, rutin, morin, quercetin, caffeic acid and santalbic acid among others (Mojirayo *et al*., 2015; Wisdom *et al*., 2016; Adeoluwa *et al*., 2019; Ahmad *et al*., 2021). Saliu & Olabiyi (2016) reported that *O. subscorpioidea* Oliv. exhibited promising inhibitory effects against acetylcholinesterase and butyrylcholinesterase and as such can be employed in the management of Alzheimer's disease. Ethanol extract of the plant was reported to demonstrate antihyperlipidemic activity (Gbadamosi *et al*., 2017), while ethanol leaf extract reportedly possesses antidepressant activity (Adeoluwa *et al*., 2015).

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 male sexual function (MSF)-enhancement potential of AREOS and also determine its phytochemistry and toxicity on the liver and kidney of wistar rats.

MATERIALS AND METHODS

Plant material

O. subscorpioidea Oliv. was obtained from Shere hills in Lamingo area of Jos, Nigeria. It was identified and authenticated at the Herbarium Unit of the Department of Plant Science and Biotechnology, University of Jos, Nigeria, where a voucher specimen was deposited and voucher number obtained (UJH000312).

Experimental animals

Eighty (50 males and 30 females) Wistar rats of average weight 120 ± 20 g (male) and 110 ± 20 g (female) 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 University of Jos Institutional Animal Care and Use Committee, and the research was allocated reference number UJ/FPS/F17-00379.

Drugs, assay kits and reagents

Drugs used include clonidine hydrochloride tablet (Lot No. 252440) and Adam's desire (Lot No.1746197), which were products of Sandoz Limited, Surrey, England and Now Foods, USA respectively. Premarin (Lot No. L95448) and Gestron-25 (Lot No. 150638) were products of Pfizer limited, Kent, United Kingdom and NTMC, Ningbo, P. R. China respectively. Assay kit for DHT was a product of Elabsience Biotechnology Co., Ltd, Houston, Texas, USA. Kit 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 and AST were products of Fortress Diagnostics Limited, Antrim, United Kingdom, while that of albumin was a product of Pointe Scientific, Inc. Canton, USA. Nitric oxide was determined using a kit manufactured by Oxford Biomedical Research Inc., USA

Preparation of extract

The fresh roots of *O. subscorpioidea* Oliv. were collected, cut into small parts and spread on a laboratory table to air-dry at room temperature (24 ^{o}C) until constant weight was achieved. The plant materials were then transferred to an oven (Carbolite PF 200, Keison Products, Essex, United Kingdom) set at 40 ^{o}C for 10 min before being reduced into fine powder with the aid of a hammer mill (Model PC 200 x 300, DEWO Machinery Company Ltd, Zhengzhou, Henan, China). Exactly 2000 g of the powdered plant material was extracted with 5000 mL of distilled water for 48 h at 4 ^{o}C (Haier Thermocool, model HRF-185BLUX, HPZ Nigeria Ltd, Ilupeju, Lagos, Nigeria) with intermittent stirring. The mixture was thereafter filtered using Whatmann No. 1 filter paper and the filtrate concentrated to dryness in an oven at 40 ^{o}C. The extract was reconstituted in distilled water to give the required doses used in this study.

Secondary metabolite analysis

The screening of *O. subscorpioidea* Oliv. sample for saponins, phenolics, flavonoids, steroids, coumarins, glycosides, terpenoids, tannins, triterpenes, anthocyanins, phlobatannins and alkaloids was done by adopting the methods described by Egbuna *et al*. (2019). The detected secondary metabolites (i.e. saponins, phenolics, flavonoids, steroids, coumarins, glycosides and terpenoids) were quantified by the methods described by Egbuna *et al*. (2019).

Induction of MSD

Mature male and female Wistar rats were first subjected to sexual training, which was done by pairing male rats with female counterparts and observing them for sexual behaviours. Rats that showed sexual experience were recruited for this test. Sexual dysfunction was induced in the sexually experienced male rats by a single oral dose of 0.5 mg/kg BW of clonidine. Sexually experienced female rats were made receptive by sequential subcutaneous administration of 10 µg/100 g BW of oestradiol conjugate (Premarin) and intramuscular administration of 0.5 mg/100 g BW of progesterone (Gestron-25) 48 h and 4 h respectively before pairing (Amin *et al*., 1996). Oestrous phase in female rats was confirmed by vaginal smears examinations according to OECD-406/407 guidelines (Steinberg *et al*., 2019). Male animals were paired with receptive female ones in ratio 1:1. Sexual behaviour parameters presented in Table II were monitored using the methods of Amin *et al*. (1996) and Agmo (1997). Male rats which showed minimum of 25% reduction in Mount frequency (MF), intromission frequency (IF) and ejaculation frequency (EF) as well as minimum increase of 25% in mount latency (ML), intromission latency (IL), ejaculation latency (EL) and post ejaculatory interval (PEI) were considered sexually impaired and recruited for the subsequent study (Malviya *et al*., 2011).

Animal grouping and extract administration

To evaluate the effect of AREOS on biochemical parameters of MSF, thirty (30) sexually matured male rats were randomly assigned into 6 groups (i. e. A, B, C, D, E, and F) each containing 5 rats. Group A represented control in which SD was not induced and administered distilled water only while groups B - F represented clonidine-induced sexually dysfunctional rats administered distilled water, Adams desire (13.3 mg/kg BW) and AREOS at 250, 500 and 1000 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 male reproductive hormones and nitric oxide.

For the toxicity evaluation, 20 male rats (not treated with clonidine) were grouped into W, X, Y, and Z, each group containing 5 rats, and administered distilled water, and AREOS at 250, 500, and 1000 mg/kg BW respectively. The rats were treated for 7 days and sacrificed 24 h after the last treatment administration. They were anaesthetized by exposing them to a cotton wool soaked with diethylether in an air-tight desiccator and thereafter sacrificed by jugular puncture. Their blood and livers were collected and prepared for analysis of aspartate and alanine transaminases activities and total protein and albumin concentrations.

Preparation of serum and tissue homogenate

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 and liver samples excised from the rats were immediately 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, and supernatants collected into sample containers for determination of the selected biochemical parameters.

Determination of biochemical parameters

Biochemical assays were done using an absorbance microplate Reader (SpectraMax 340PC384, Molecular Devices, San Jose, California, USA). The methods described by Tietz (1995) were used for the determination of testosterone, AST, ALT, and total protein. LH was determined by the method of Cumming *et al*. (1985) while NO was determined by the method of Schmidt (1995). The method described by Sartorius *et al*. (2014) was employed for the assay of DHT while albumin was determined by the method of 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 *O. subscorpioidea* Oliv.

The secondary metabolites constituents of AEOSR are presented in Table I. Glycoside had the highest concentration (294.35 ± 0.83 mg/100 g) while phenolics had the lowest (4.96 ± 0.00 mg/100 g). Tannins, triterpenes, anthocyanins, phlobatannins and alkaloids were not detected.

Table I: Secondary metabolites constituents of aqueous root extract of *O. subscorpioidea* Oliv.

Secondary metabolite	Concentration (mg/100g)
Saponins	63.23 ± 0.17
Phenolics	4.96 ± 0.00
Flavonoids	16.86 ± 0.00
Steroids	33.9 ± 02.81
Coumarins	24.93 ± 1.03
Glycosides	294.35 ± 0.83
Terpenoids	18.96 ± 0.02
Tannins	Not detected
Triterpenes	Not detected
Anthocyanins	Not detected
Phlobatannins	Not detected
Alkaloids	Not detected

Values are means ± SD (of 2 determinations)

Amino acid composition of aqueous root extract of *O. subscorpioidea* Oliv.

Eighteen of the twenty standard amino acids were detected in AEOSR. Glutamic acid had the highest concentration (11.90 mg/100g protein) while methionine was lowest at 0.91 mg/100g protein (Table II). Asparagine and glutamine were not detected as they were converted to aspartic acid and glutamic acid respectively during acid hydrolysis.

Table II: Amino acid composition of aqueous root extract of *O. subscorpioidea* Oliv.

Amino Acid	Concentration (mg/100 g protein)
Leucine	5.02 ± 0.12
Lysine	4.20 ± 0.02
Isoleucine	4.80 ± 0.04
Phenylalanine	4.25 ± 0.11
Tryptophan	3.31 ± 0.12
Valine	3.60 ± 0.01
Methionine	0.91 ± 0.01
Proline	3.04 ± 0.02
Arginine	5.33 ± 0.32
Tyrosine	3.10 ± 0.14
Histidine	3.13 ± 0.11
Cystine	2.06 ± 0.13
Alanine	3.20 ± 0.15
Glutamic acid	11.90 ± 0.02
Glycine	5.01 ± 0.15
Threonine	4.00 ± 0.11
Serine	3.94 ± 0.23
Aspartic acid	9.70 ± 0.32

Values are means ± SD (of 2 determinations).

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Confirmation of induction of male sexual dysfunction

The effect of clonidine administration on male sexual behaviour parameters in rats is presented in Table III. MF, IF, and EF reduced by 40.00, 36.78, and 31.30 % respectively, while ML, IL, EL, and PEI respectively increased by 32.99, 33.05, 48.78 and 34.84.

Table III: Male sexual function parameters in rats exposed to clonidine

Parameters	Control (Distilled water)	Clonidine-treated (0.5 mg/kg BW)	Percentage change
Mount frequency	1.25 ± 0.12	0.75 ± 0.03	40.00↓
Intromission frequency	4.35 ± 0.11	2.75 ± 0.02	36.78↓
Ejaculation frequency	16.20 ± 0.13	11.13 ± 0.12	31.30↓
Mount Latency (sec)	20.55 ± 0.22	27.33 ± 0.13	32.99↑
Intromission Latency (sec)	20.85 ± 0.31	27.74 ± 0.02	33.05↑
Ejaculation Latency (sec)	0.82 ± 0.01	1.22 ± 0.11	48.78↑
Post ejaculatory interval (sec)	12.43 ± 0.42	16.76 ± 0.21	34.84↑

Key= ↓ (percentage decrease), ↑ (percentage increase), Control values are means (of 5 replicates) ± Standard Error of Mean, while clonidine-treated values are means (of 30 replicates) ± Standard Error of Mean

Hormonal profile and nitric oxide concentration

Hormonal profile and NO concentration in rats administered AEOSR are presented in Table IV.

Testicular testosterone level was significantly increased ($p < 0.05$) in rats administered clonidine when compared with the sham control. AEOSR at all doses significantly reduced ($p < 0.05$) testicular testosterone level when compared with the clonidine plus distilled water-treated and clonidine plus Adam's desire-treated groups. Only the group treated with AEOSR at 1000 mg/kg BW compared favourably ($p > 0.05$) in testicular testosterone concentration with the sham control group (Table IV).

Similarly, clonidine administration significantly elevated ($p < 0.05$) serum testosterone level when compared with the sham control group (Table IV). AEOSR and Adam's desire reduced ($p < 0.05$) serum testosterone level when compared with clonidine plus distilled water-treated group. All AEOSR-treated rats produced a significantly lower ($p < 0.05$) serum testosterone level than the clonidine plus Adam's desire-treated rats. Rats administered AEOSR at 1000 mg/kg BW had a significantly lower ($p < 0.05$) serum testosterone level than those administered AEOSR at 250 and 500 mg/kg BW (Table IV).

Conversely, clonidine administration significantly reduced ($p < 0.05$) serum LH level when compared with the sham control (Table IV).

There was no significant difference ($p > 0.05$) in serum LH level of rats administered clonidine plus distilled water, clonidine plus Adam's desire and clonidine plus AEOSR at 1000 mg/kg BW (Table IV). Only rats administered AEOSR at 250 and 500 mg/kg BW showed serum LH levels comparable ($p > 0.05$) with the sham control group (Table IV).

Table IV: Hormonal profile and nitric oxide concentration in clonidine-treated male rats administered aqueous extract of *O. subscorpioidea* Oliv. root.

Treatment	Testicular testosterone (pg/ml)	Serum testosterone (pg/ml)	Luteinizing hormone (mIU/ml)	Follicle stimulating Hormone (mIU/ml)	Dihydrotestosterone (ng/l)	Nitric oxide (µM)
Sham control (Distilled water)	28.44 ± 0.52^a	4.84 ± 0.32^a	3.84 ± 0.65^a	3.92 ± 1.10^a	2.21 ± 0.11^a	20.97 ± 0.52^a
Clonidine + Distilled water	58.24 ± 0.41^b	37.62 ± 1.17^b	1.78 ± 0.54^b	1.22 ± 0.52^b	3.50 ± 0.10^b	19.74 ± 0.70^a
Clonidine + Adam's desire (1.5 mg/kg BW)	51.88 ± 0.97^c	25.00 ± 1.44^c	1.28 ± 0.21^b	1.15 ± 0.60^b	3.12 ± 0.16^b	10.15 ± 0.71^b
Clonidine + AEOSR (250 mg/kg BW)	45.05 ± 0.67^d	12.87 ± 0.27^d	3.55 ± 1.56^a	1.36 ± 0.12^b	2.55 ± 0.74^a	15.76 ± 0.78^c
Clonidine + AEOSR (500 mg/kg BW)	44.95 ± 0.97^d	12.51 ± 0.67^d	3.25 ± 1.51^a	1.36 ± 0.12^b	2.45 ± 0.22^a	15.76 ± 0.78^c
Clonidine + AEOSR (1000 mg/kg B.W)	30.48 ± 0.66^a	1.74 ± 0.48^a	1.19 ± 0.24^b	1.27 ± 0.06^b	2.64 ± 0.16^a	13.21 ± 1.67^c

Values represent mean ± SEM; $n = 5$; values with different superscripts down the column are significantly different ($p < 0.05$); AEOSR: Aqueous Extract of *O. subscorpioidea* Oliv. Root

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Clonidine administration significantly reduced ($p < 0.05$) serum FSH level when compared with the sham control group (Table IV). Rats treated with AEOSR (at all doses) and Adam's desire did not differ significantly ($p > 0.05$) in serum FSH concentration when compared with the clonidine plus distilled water-treated rats (Table IV).

Clonidine significantly increased ($p < 0.05$) serum DHT concentration when compared with the sham control rats (Table IV). AEOSR (at all doses) significantly reduced ($p < 0.05$) serum DHT level when compared to the clonidine plus distilled water-treated group. There was no significant difference ($p > 0.05$) in serum DHT level of the AEOSR-treated groups and the sham control (Table IV).
Clonidine administration did not alter ($p > 0.05$) serum NO level when compared with the sham control group (Table IV). AEOSR and Adam's desire significantly reduced ($p < 0.05$) serum NO level when compared with sham control and clonidine plus distilled water-treated groups (Table IV).

Liver function indices
Liver and serum aminotransferases activities in rats administered AEOSR is presented in Table V.
Exposure to AEOSR at the various doses tested did not alter ($p > 0.05$) liver ALT activity when compared with the sham control. However, serum ALT activity was significantly reduced ($p < 0.05$) in the AEOSR-treated rats when compared with the sham control. AEOSR-treated rats at 1000 mg/kg BW had a significantly higher ($p < 0.05$) serum ALT activity than rats administered AEOSR at 250 and 500 mg/kg BW) (Table V).
Administration of AEOSR at all doses did not cause any significant alteration ($p > 0.05$) to liver and serum AST activity in rats when compared with the distilled water-treated sham control (Table V).
Serum total protein and albumin concentrations in rats administered AEOSR are presented in Table VI. Serum total protein and albumin levels did not differ significantly ($p > 0.05$) in the AEOSR-treated rats from the levels recorded in the distilled water-treated sham control rats. AEOSR at 1000 mg/kg BW produced a significantly higher ($p < 0.05$) serum total protein concentration than AEOSR at 250 and 500 mg/kg BW (Table VI).

Table V: Liver and serum transaminases activities in rats administered aqueous root extract of *O. subscorpioidea* Oliv

Group	Liver ALT (U/L)	Serum ALT (U/L)	Liver AST (U/L)	Serum AST (U/L)
Sham control (Distilled water)	345.92 ± 17.63^a	71.45 ± 3.96^a	301.97±11.58^a	195.79 ± 11.73^a
AEOSR (250 mg/kg BW)	333.25 ± 20.32^a	49.55 ± 6.94^b	305.55 ± 2.33^a	180.87 ± 9.11^a
AEOSR (500 mg/kg BW)	321.45 ± 21.87^a	48.64 ± 7.04^b	311.71 ± 3.50^a	179.87 ± 7.49^a
AEOSR (1000 mg/kg BW)	361.84 ± 29.19^a	57.37 ± 11.99^b	308.16 ± 3.91^a	189.87 ± 8.17^a

n = 5 ± SEM; Values carrying superscripts different from the control down the column are significantly different at $p < 0.05$. ALT = alanine aminotransferase, AST = aspartate aminotransferase, AEOSR = Aqueous root extract of *O. subscorpioidea* Oliv.

Table VI: Sub-acute effect of aqueous root extract of *O. subscorpioidea* Oliv. on serum concentrations of total protein and albumin in Wistar rats

Group	Total protein (g/dl)	Albumin (g/dl)
Sham control (Distilled water)	7.69 ± 0.48^{ab}	5.14 ± 0.23^a
AEOSR (250 mg/kg BW)	6.82 ± 0.29^a	4.70 ± 0.42^a
AEOSR (500 mg/kg BW)	6.71 ± 0.09^a	4.51 ± 0.38^a
AEOSR (1000 mg/kg BW)	8.39 ± 0.45^b	4.80 ± 0.25^a

n = 5 ± SEM; Values carrying superscripts different from the control down the column are significantly different at $p < 0.05$. ALT = alanine aminotransferase, AST = aspartate aminotransferase, AEOSR = Aqueous root extract of *O. subscorpioidea* Oliv.

DISCUSSION
Plant secondary metabolites are responsible for the pharmacological and toxicological activities they exhibit (Zeeshan *et al*., 2022). The secondary metabolites of AEOSR based on our analysis align with those previously reported in the plant's stem and root (Ayandele & Adebiyi, 2007; Ukwe *et al*., 2010). Plant secondary metabolites such as saponins and flavonoids have been reported to impact on the biosynthesis of testosterone and other male reproductive hormones (Saeed *et al*., 2024). Flavonoids like quercetin, epigallocatechin-3-gallate, and rutin among others have demonstrated ability to protect and improve the hypothalamic-pituitary-testicular health thus aiding spermatogenesis and steroidogenesis and as a result bringing about improvement in male reproductive and sexual functions (Mishra *et al*., 2024). The ability of AEOSR to normalize clonidine-mediated alterations in rat male sex hormones can be attributed to the secondary metabolites present in the extract.

In this study, amino acid analysis revealed that glutamic acid and aspartic acid were relatively higher in AEOSR than other amino acids. This is attributable to the conversion of glutamine and asparagine to glutamic acid and aspartic acid respectively by the

acid hydrolysis step of the analysis. Amino acids such as glutamine, arginine, tryptophan, tyrosine, glycine, and phenylalanine, which were all detected in AEOSR, have all been implicated to play roles in male sexual functions. Arginine, which is the third most abundant amino acid in AEOSR, serves as a precursor for the biosynthesis of nitric oxide, which increases arterial elasticity, reduces blood pressure and improves erectile processes, stamina and sexual performance (Rhim *et al*., 2019). Phenylalanine and L-tyrosine are precursors in the synthesis of L-3, 4-dihydroxyphenylalanine (L-DOPA), dopamine, norepinephrine, and epinephrine (adrenaline), all of which are potential compounds for enhancing libido (Kayode & Yakubu, 2017). Tryptophan is hydroxylated to serotonin, which may induce both inhibitory and regulatory effects on male sexual function, influencing arousal, ejaculation and overall sexual satisfaction depending on its levels and receptors involved (Hull *et al*., 2004). In addition, cysteine, glutamic acid, and glycine are key precursors for the synthesis of glutathione, a powerful antioxidant that plays a critical role in protecting cells from oxidative stress and the development of various diseases, including those related to sexual health (McCann & Maguire-Zess, 2021).

Clonidine has been employed for experimental induction of male sexual dysfunction (MSD) by a couple of researchers (Srilatha *et al*., 1999; Omoniwa *et al*., 2022). In this study, male Wistar rats exposed to clonidine exhibited a percentage reduction in MF, IF, and EF and a percentage elongation of ML, IL, EL, and PEI to degrees exceeding the 25 % benchmark for MSD induction.
Serum and testicular testosterone and serum DHT were elevated in clonidine-treated sexually dysfunctional male rats. Similar result was documented by Lin *et al*. (2015), where clonidine induced sexual dysfunction in male rats but did not alter the levels of testosterone, FSH, and LH in spontaneously hypertensive male rats. This underscores the importance of factors other than male sex hormones and the imperativeness of having normal concentrations of sex hormones in the facilitation of sexual response in males. Administration of AEOSR caused a reduction in testosterone and DHT levels. The normalizing action of AEOSR on testosterone level was dose-dependent with the 1000 mg/kg BW dose performing better than the lower doses. Normal levels of testosterone and DHT are required for proper sexual functions (Kohn *et al*., 2017). Many plant extracts and phytochemicals have elicited modulatory properties on androgen biosynthesis and thus male sexual functions and fertility (Mishra *et al*., 2024; Saeed *et al*., 2024). For example, a combination of *Mentha spicata* L. and *Linum usitatissimum* L. demonstrated testosterone-lowering activity in female rats with PCOS (Mehraban *et al*., 2020). LH and FSH levels were reduced by clonidine administration in this study. LH and FSH work synergistically in reproduction. While FSH plays a very crucial role in pubertal maturation and spermatogenesis, LH stimulates the synthesis and secretion of testosterone (Grinspon *et al*., 2018; El Sayed *et al*., 2023). Normally, elevated testosterone level directly correlates with elevated LH, however, this was not the case following clonidine administration. The recorded reduction in LH and FSH may be attributed to negative feedback effect on their biosynthesis by the elevated testosterone. Although AEOSR did not normalize FSH level, it restored LH level to normal level at the 250 and 500 mg/kg BW doses. Bioactive compounds in AEOSR may possess activities that counteract testosterone inhibitory effect on gonadotropins production. Adams Desire could not compare favourably with the extract at all the doses examined. It only made at attempt to reduce clonidine-mediated elevation of serum and testicular testosterone, but did not have any effect on the abnormal levels of LH, FSH, and DHT. AEOSR on the other hand restored testicular testosterone (at 1000 mg/kg BW), LH (250 and 500 mg/kg BW) and DHT (all doses) to control levels.

NO action in relation to male sexual function involves the stimulation of guanylate cyclase, the enzyme that catalyses the formation of cyclic GMP, which initiates a number of biochemical reactions that culminates in smooth muscle relaxation and penile erection (Surks, 2007; Ball *et al*, 2012). Clonidine did not alter NO synthesis in this study, but AEOSR at all the doses examined caused a reduction in NO concentration. It is possible that some secondary metabolites in AEOSR may inhibit NO synthase activity or repress its synthesis (Liu *et al*., 2023) while arginine which is present in AEOSR may have been acted upon by arginase rather than being converted to NO by NO synthase (Clemente *et al*., 2020). This indicates that the influence of AEOSR on male sexual functions may be selective and not involve activation of NO synthase, the enzyme that catalyses NO synthesis. Previous studies have revealed that plant extracts may enhance or dampen NO synthesis. For example Kayode and Yakubu (2017) reported the enhancement of NO synthesis in paroxetine-induced sexually dysfunctional rats following treatment with aqueous leaf extract of *Parquetina nigrescens* while extracts of *Solanum melongena* and *Solanum macrocarpon* were reported to inhibit NO production in RAW 264.7 cells (Ng *et al*., 2015).

One of the functions of the liver is xenobiotic metabolism and detoxification (Kalra *et al*., 2024). Enzymes which are predominantly found in the liver and other metabolites produced as a result of the functioning of the liver can be monitored to ascertain whether the liver is functioning properly or not (Lala *et al*., 2024). AEOSR did not cause any derangement of liver cells membrane as indicated by the unaltered liver ALT and liver and serum AST activities. The recorded reduction in serum ALT activity may be a pointer to potential repression of ALT biosynthesis or inhibition of its activity by AEOSR which however had not reflected in the liver.

Total protein, albumin, and globulin are proteins produced by the liver and their blood levels indicate whether the liver's synthetic function is intact or otherwise (Lala *et al*., 2024). While AEOSR did not alter serum albumin level, total protein was elevated in the group exposed to the highest dose of AEOSR (1000 mg/kg BW). Since total protein represents the summation of albumin and globulin, and albumin level was not altered, it is safe to infer that AEOSR at 1000 mg/kg BW may induce excessive production of globulin or increased humoral immune activity (Hashash *et al*., 2022). An elevation of globulin level may be an indication of infection, dehydration, underlying inflammation, or liver-related conditions, and may lead to an increase in blood viscosity, which may strain the cardiovascular system (Nader *et al*., 2019).

Conclusion

In conclusion, AEOSR exhibits promising potential for managing clonidine-induced male sexual dysfunction through its bioactive secondary metabolites, including flavonoids and saponins, which positively affect testosterone, DHT, and LH levels. Although AEOSR did not reverse clonidine's inhibition of FSH or NO synthesis, it selectively supported reproductive health without liver toxicity. Elevated globulin levels at high doses suggests immune-

modulatory effects, possibly enhancing humoral immunity. These findings underscore AEOSR as a viable natural option for enhancing male sexual health, though further studies are necessary to evaluate its effects on immune and cardiovascular health in relation to globulin elevation.

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