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The Effectiveness of Combined Ethanol Root Extract of *Terminalia avicennioides, Terminalia superba* and Seeds of *Hunteria umbellata* on Paroxetine-Induced Erectile Dysfunction in Male Wistar Rats

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ABSTRACT: Erectile dysfunction (ED) has posed problems among several married couples. This study evaluated the efficacy of combined ethanol root extracts of *Terminalia avicennioides*, *Terminalia superba* and seeds of *Hunteria umbellata* on Paroxetine-induced erectile dysfunction in male Wistar rats. Fresh roots of plants were collected, pulverized, extracted with 70% ethanol, dried in an oven at 40^0C and subjected to phytochemical screening. The plants were mixed in a ratio of 1:1:1; *T. superba* and *T. avicennioides* (TASM) mixed; *T. superba*, *T. avicennioides* and *H. umbellata* (TASHM) mixed. The rats were acclimatized, grouped and induced with paroxetine hydrochloride at a dose of 10 mg/kg. Sildenafil was used as a standard control at a dose of 10 mg/kg. The rats received 1 ml of TASM and TASHM. Nitric oxide (NO), male sex hormones and histological examinations of testes were evaluated. Saponins reducing sugars, terpenoids, steroids, phenolic compounds, tannins, and flavonoids were present in all the plant extracts. NO showed a significant increase ($p<0.05$) at 75 mg/kg body weight of TASM and TASHM, while LH, FSH, and TH presented a significant ($p<0.05$) increase at 100 mg/kg of TASHM. Increased level of spermatogenesis and Leydig cell proliferation were evident in histology studies. A combination of TASHM at 75 mg/kg produced moderate efficacy.

Keywords: Erectile dysfunction, *Terminalia avicennioides, Terminalia superba, Hunteria umbellata*, Nitric oxide.

Introduction

There is a general belief that couples have problems with conception, despite regular unprotected sexual intercourse for at least twelve months and this could be a problem from either of the partners or both (Polis *et al*., 2020). Erectile dysfunction (ED) appears to be a widespread sexual issue in men leading to the problem of infertility in marriages (Marinelli *et al*., 2021). Craig *et al*., (2017) defined ED as a man's inability to achieve or maintain an erection of sufficient rigidity for vaginal penetration and completion of a sexual act. ED is a prevalent health challenge that has a considerable impact on the quality of life of middle-aged men (Esposito *et al*., 2015). Several causes of ED have been discovered to be psychological and physiological, among which are endocrine disorders, stress, depression, anxiety, age, pelvic surgery, lifestyle, obesity, as well as diseases such as diabetes, multiple sclerosis, hypertension and heart related conditions (Elkhoury *et al*., 2017; Bruening *et al*., 2019; Nicolini *et al*.,2019; Masuku *et al*., 2020). Medications such as antidepressants, tranquilizers, hypnotics, anti-androgens and anti-hypertensive agents also contribute to ED (Reed-Maldonado and Lue, 2016). Age-related erectile dysfunction is associated with multifactorial alterations in the cavernosal endothelial cell lining, smooth muscle cells and synthesis of nitric oxide (Bruening *et al*., 2019). The mechanism of ED borders on the formation of penile tissue by two dorsal corporal bodies known as corpora cavernosa, composed of sinusoidal spaces lining the endothelium of the penile tissue (Bruening *et al*., 2019). Neural transmitters such as acetylcholine, are released from the cavernosal nerve endings and stimulate the neuronal nitric oxide synthase (nNOS) to catalyze the release of nitric oxide (NO) from the endothelium (Diaber *et al*., 2019), which increases cyclic guanosine monophosphate (cGMP) levels. cGMP causes the smooth muscle to relax, which causes an inflow of blood, leading to erection (Onal *et al*., 2016). The inhibition of NOS increases superoxide (O2-) production in penile arteries (Kaya *et al*., 2017). As individuals age, free radicals of O2- are produced at a higher rate, which causes ineffective relaxation of cavernosal tissues (Alwaal *et al*., 2015). Several management measures of ED include modulation of phosphodiesterase 5 (PDE5) activity, which enzymatically converts the intracellular second messenger molecule cGMP to its inactive form e.g sildenafil citrate, propionyl-l-carnitine, dehydroepiandrosterone, L-arginine (Alwaal *et al*., 2015; Reed-Maldonado and Lue, 2016). Gene therapy for PDE5-resistant ED (Yu *et al*., 2018), modulation of enzymes associated with NOS/cGMP pathway as well as supplementation of trophic factors, peptides and potassium ion channels have also been employed (Reed-Maldonado and Lue, 2016). More so, lifestyle changes such as regular exercise, healthy feeding, abstinence from smoking, reduction of alcohol intake, stress reduction, continued sexual activities and use of certain medicinal plants have provided some degree of management measures (Nurudeen *et al*., 2015). *T. avicennioides* of the family Combretaceae, has been used for the management of many pathological conditions among most inhabitants of rural communities in Nigeria and many other African countries (Yahaya *et al*., 2019). The parts of the plant mostly used for medicinal purposes are roots and stem barks, which are rich sources of phytochemicals such as flavonoids, saponins, steroids, tannins and terpenes among others (Azeez *et al*., 2017). In folk medicine, claims have been made about this plant being used to cure various diseases such as dental caries, skin infections, ulcers, syphilis, bloody sputum, ringworm infection, gastrointestinal helminth and diabetes (Aiyelaagbe *et al*., 2014; Dzubak *et al* 2016; Hussein and El-Anssary, 2018; Khameneh *et al*., 2019; Yahaya *et al*., 2019).

H. umbellata (K. Schum) Hallier f. of the family Apocynaeceae is a tropical rainforest tree which is popularly used for the local management of certain diseases such as diabetics mellitus, obesity, hypertension and infertility (Adeyemi *et al*., 2011; Patra *et al*., 2015; Adeneye *et al*., 2019; Srivatsay *et al*., 2020; Akinrotoye *et al*., 2020; Fadahunsi *et al*., 2021). *T. superba* belongs to the family Combretaceae, widely distributed in Africa and known to contain xanthones, triterpenoids, lignans, saponins, cardenolides, flavonoids, steroids, phenolics and tannins (Bamisaye *et al*., 2019; Zhang *et al*., 2019). *T superba* has been reportedly used for the treatment of diabetes, infertility, abdominal pains, bacterial, fungal and viral infections (Ahon *et al*., 2011; Tom *et al*., 2011; Tom *et al*., 2014; Désiré *et al*., 2014). GC-MS analysis of the seed of *H. umbellata* has confirmed the presence of 2-2/-Benzylidenebis (3 methylbenzofuran), Benzenemethanol, Pheno,6-methoxy-2[2-(2-quinoly) etheny, 2-Ethylacridine, Glibenclamide, 11-Octadecenoic acid, Urs-12-en-24-oic acid and 9-Octadecenoic acid (2) (Ladokun *et al*., 2018).

This research objective plans to identify the capability of using a combined part of these three plants in the treatment of erectile dysfunction, to bring cheap and effective treatment to men who are challenged with ED.

Materials and methods

Collection and authentication of plant materials: Some fresh roots of *T. superba*, *T. avicennioides* and seeds of *H. umbellata*, were harvested from a farmland at Mushin in Lagos State, Nigeria. The plants were authenticated at the herbarium Unit of the Department of Botany, University of Lagos, Nigeria and the respective voucher specimens (LUH 8492, LUH 8493 and LUH 8494) were submitted for future reference.

Experimental animals: Twenty-seven healthy matured male rats (138.30 kg ± 59.0 kg) of Wistar strain were used for this study. The animals were maintained in the well-ventilated Animal House located within Biochemistry Department, Nigerian Institute of Medical Research (NIMR) Yaba, Lagos State, under the housing conditions of temperature: 20±2 °C; photoperiod of about 9-11 h light and dark; and relative humidity of 45-55 % as guidelines of Organization for Economic Co-operation and Development (Bhandari *et al*., 2021). The animals were acclimatized for one week and were fed on rat pellets and clean tap water throughout the experiment.

Preparation of aqueous extracts: The roots of *T. superba* and *T. avicennioides* were sliced into tiny pieces and dried under shade in the Department of Cell Biology and Genetics Laboratory, University of Lagos Nigeria, for one week. The dried root samples weighed 215 and 98.6 g respectively, while the seeds of *H. umbellata* weighed 99.7 g after pulverization. Fifty grammes of each pulverized plant were soaked in 500 ml 70 % ethanol for 3 days. The filtrate was concentrated using a rotary evaporator, oven dried at 40 °C and yielded crude dried extracts weighing 6.0, 11.39 and 10.44 g respectively.

Plant extract mixtures: The three plants used were mixed in the ratio of 1:1:1 as follows; *T. superba* was mixed with *T. avicennioides* (TASM). While *T. superba* and *T. avicennioides* were further mixed with *H. umbellata* (TASHM). Three different doses of 50, 70 and 100 mg/ kg were prepared for each of the two groups. Each extract group TASM and TASHM received 0.05, 0.075 and 0.1 ml of the extract for every 1 kg of the rat respectively.

Induction of sexual dysfunction in the male rats and grouping of animals: All the experimental rats weighing between (80-101 kg) were fed *ad libitum* with rat pellets purchased from the Animal House located within NIMR and given 0.5 ml of distilled water before the commencement of the experiment. A total of 27 male rats were used for this study and were acclimatized for one week. Paroxetine hydrochloride (10 mg/kg) was used to induce erectile dysfunction in the male experimental rats, prepared by dissolving 20 mg in 20 ml of distilled water. One millilitre of paroxetine was used to induce erectile dysfunction in the experimental rats. The animals were divided into four groups namely; negative, positive, standard and treated groups. The negative, positive and standard groups had 3 rats each, while the treated group was divided into two (TASM and TASHM) each having a triplet. The negative control was not induced with paroxetine, the positive control was induced with paroxetine but not treated, the standard group was treated with paroxetine and sildenafil citrate (10 mg/kg). Animals in the treated group were induced with paroxetine and received three different doses of TASM and TASHM at 50, 70 and 100 mg/kg. The extract was administered using oral syringes via oral route to the rats for three alternate days (Day 1, Day 3, and Day 5). All procedures in this study were carried out following the Ethical Approval of the Biochemistry Department, NIMR. The animals were humanly handled according to the Guide of the Committee for the update on the care and use of Laboratory Animals in NIMR.

Phytochemical screening: Aqueous root extracts of *T. superba*, *T. avicennioides* and seeds of *H. umbellata*, were screened for the qualitative and quantitative presence of alkaloids, saponins, tannins, phenolics, phlobatanins, anthraquinones, cardenolides, cardiac glycosides, steriods, triterpenes, flavonoids, chalconoes according to the procedures described by Kumar *et al*., (2017); Abdelwahab *et al*. (2019); Munira *et al*. (2019).

Preparation of serum supernatants: On Day 6, the rats were subjected to euthanasia by cervical dislocation. The internal organs were exposed using sterile forceps and scissors. Five milligrams of blood samples were collected through the ocular sinus with a 5 ml syringe into labeled collecting tubes, the samples were centrifuged at 3000 rpm for 10 min to obtain the serum. The sera collected were stored at -4 °C for biochemical analysis.

Determination of male serum reproductive hormone concentrations: The male serum reproductive hormone concentrations were quantitatively determined following the instructions and procedures outlined in the manufacturer's manual contained in the assay kits (DiaMetra FSH ELISA SEGATE (MI) Italy)

Determination of serum nitric oxide concentration: This was done based on the fact that nitrate is reduced by copper coated cadmium and the nitrite produced is determined by diazotization of Griess reagent under acidic conditions. Griess reaction measures nitrite which reflects nitric oxide production rate (Hetrick and Schoenfisch, 2009).

Histological examination of the testes: The testes were harvested, while the tissues were placed in a fixative, 10 % Formal saline and taken to the Histology laboratory, Department of Morbid Anatomy, Obafemi Awolowo University Teaching Hospital. The procedure was carried out as described by Khalaf *et al*., (2019).

Statistical analysis: The data collected were expressed as the mean + SEM of three determinations. One-way ANOVA was done to compare the means of different groups as well as Duncan multiple range tests to analyze differences between groups. Values of $P<0.05$ were regarded as significant. Statistical Package for Social Sciences, version 16.0 (SPSS Inc, Chicago, USA) was used for the statistical analysis.

Results

Phytochemical screening: The qualitative phytochemical screening of the three plants revealed the presence of saponins reducing sugars, terpenoids, steroids, phenolic compounds, tannins, and flavonoids in all plant extracts. Anthraquinone was absent in all the plant extracts. Saponins, reducing sugars, cardiac glycosides, terpenoids, steroids, phenolic compounds, tannins, flavonoids and triterpenoids were abundant in *T. avicennioides*, while alkaloids were in trace amount. Reducing sugars, terpenoids, steroids, flavonoids and triterpenoids were abundant in *T. superba*, while saponins, phenolic compounds, cardiac glycosides and tannins were in trace amount. Alkaloids, saponins, reducing sugars, terpenoids, phenolic compounds, tannins, cardiac glycosides and flavonoids were in abundance of *H. umbellata;* steroids were in trace amount, while triterpenoid was absent.

Table 1a: Qualitative phytochemical screening of *T. avicennioides, T. superba* and *H. umbellata*

Phytochemical Assay		***T. avicennioides***	***T. superba***	***H. umbellata***
Alkaloids	Mayer's Test	Trace	_	+
	Dragendorff's Test	Trace	_	+
	Wagner's Test	Trace	_	+
Saponins	Frothing Test	+	Trace	+
Reducing Sugar	Fehling's Test	+	+	+
Anthraquinones	Bontrager's Test	_	_	_
Cardiac glycosides	Keller Killani's Test	+	Trace	+
Terpenoids	Liebermann-Burchard	+	+	+
Steroids	Salkowski's Test	+	+	Trace
Phenolic Compounds	Lead acetate Test	+	Trace	+
Tannins	Ferric chloride Test	+	Trace	+
Flavonoids	Shinoda's Test	+	+	+
Triterpenoids	Liebermann-Burchard	+	+	-

Legend: + = Abundant, - = Absent, Trace = Small amount.

In Table 1b, the quantitative phytochemical screening of the plants evaluated *T. avicennioides* (in mg/g) to contain 4.49 of alkaloids, 10.64 of saponins, 14.4 of reducing sugars, 7.82 of cardiac glycosides, 11.34 of steroids, 11.90 of phenolic compounds, 6.23 of tannins, and 16.4 of flavonoids. *T. superba* (in mg/g) contains 1.88 of saponins, 15.21 of reducing sugar**,** 0.94 of cardiac glycosides, 114 of steroids, 0.95 of tannins, 3.3 of phenolic compounds and 17.4 of flavonoids. *H. umbellata* (in mg/g) contains 59.3 of alkaloids, 15.27 of saponins, 10.16 of reducing sugar, 6.28 of cardiac glycosides, 2.13 of steroids, 11.22 of tannins, 16.32 of phenolic compounds and 6.59 of flavonoids.

Table 1b: Quantitative phytochemical screening of *T. avicennioides, T. superba* and *H. umbellata*

Phytoconstituents (mg/g)	***T. avicennioides***	***T. superba***	***H. Umbellata***
Alkaloids	4.49±0.08	-	59.33±1.77
Saponins	10.64±0.60	1.88±0.46	15.27±0.11
Reducing Sugar	14.42±0.21	15.21±0.11	10.16±0.51
Cardiac glycosides	7.82±0.76	0.94±0.40	6.28±0.20
Steroids	11.34±0.69	114.01±2.01	2.13±0.34
Tannins	6.23±0.10	0.95±0.90	11.22±0.45
Phenolic compounds	11.90±0.21	3.30±0.21	16.32±0.25
Flavonoids	16.43±0.23	17.40±0.23	6.59±0.45

Nitric Oxide Concentration (NO) Concentration with TASM: The concentration of NO among groups treated with 50 mg/kg, 75 mg/kg, and 100 mg/kg of TASM was compared with the control in Figure 1. There was a significant increase among means ($p<0.05$) in the concentration of NO group treated with 75 mg/kg of TASM. Groups treated with 100 mg/kg of TASM also showed a high level of comparison to the control. The NO concentration was significantly different ($P<0.05$) in all groups of rats treated with TASM

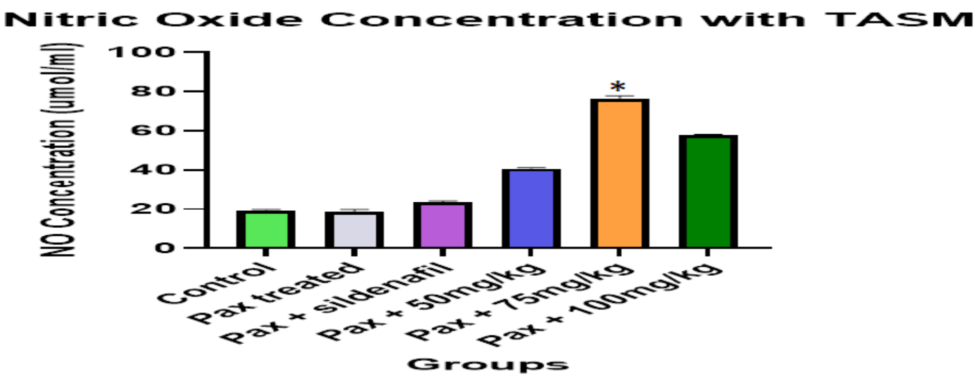

Figure 1: Variation in serum Nitric Oxide Concentration treated with TASM (*Terminalia avicennioides* and *Terminalia superba* mixed) at different doses.

Legend: Control = negative control, not induced with paroxetine. Pax = all groups induced with paroxetine

NO concentration with TASHM: NO concentration of the group treated with paroxetine reduced compared with the group not induced but given distilled water. The group treated with 75 mg/kg TASHM maintained a significant increase (*P*<0.05) among mean groups, as seen in Figure 2. Sildenafil recorded higher values in comparison with 50 mg/kg and 100 mg/kg treated groups. The group treated with 75 mg/kg and 100 mg/kg of TASHM still maintained a level of significance (*P*<0.05)

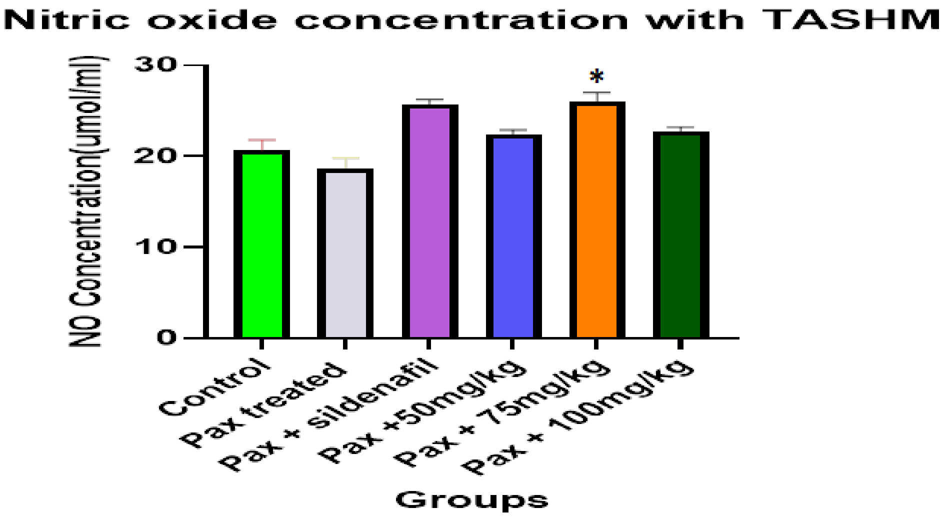

Figure 2: Variation in serum nitric oxide concentration treated with TASHM (*Terminalia avicennioides*, *Terminalia superba* and *Hunteria umbellata* (mixed) at different doses.

Legend: Control = negative control, not induced with paroxetine. Pax = all groups induced with paroxetine

Hormonal concentrations: The hormonal concentrations such as luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (TH) of the rats in different treated groups were compared. In Figure 3, the concentrations of LH in the serum of the rat group treated with 50 mg/kg of TASM was significant (*P*<0.05) among other mean groups. It was observed that all treated groups recorded higher levels of LH when compared with the standard and control groups. A similar level of significance (*P*<0.05) in the group treated with 50 mg/kg of TASM was also observed in the serum concentration of FSH (Figure 4).

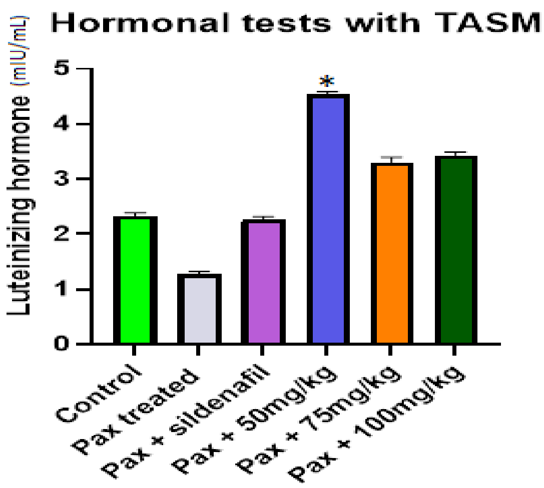

Figure 3: Variation in serum LH treated with TASM

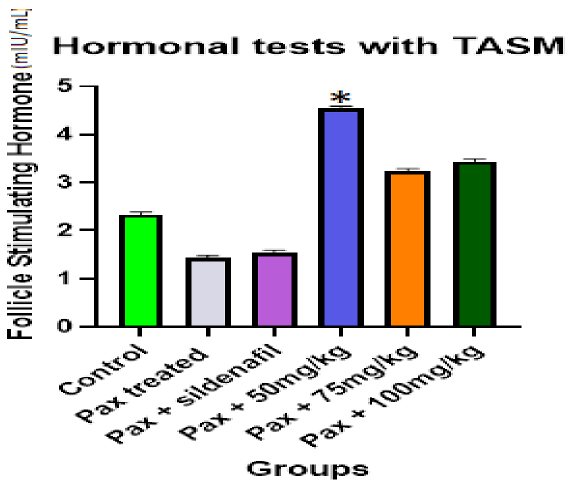

Figure 4: Variation in serum FSH treated with TASM

The level of TH significant (*P*<0.05) in the group treated with 100 mg/kg of TASM among the mean values of other groups (Figure 5).

In Figure 6, the treated groups had a significant decrease (*P*<0.05) in LH serum concentration compared with the control and standard groups. Figure 7 shows a significant decrease (*P*<0.05) in FSH serum concentrations, among the mean values of the treated groups and control. A similar level of significance (*P*<0.05) was observed in the serum concentration of TH (Figure 8).

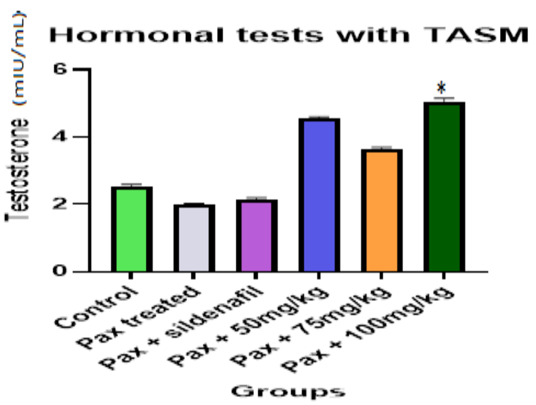

Figure 5: Variation in serum TH treated with TASM

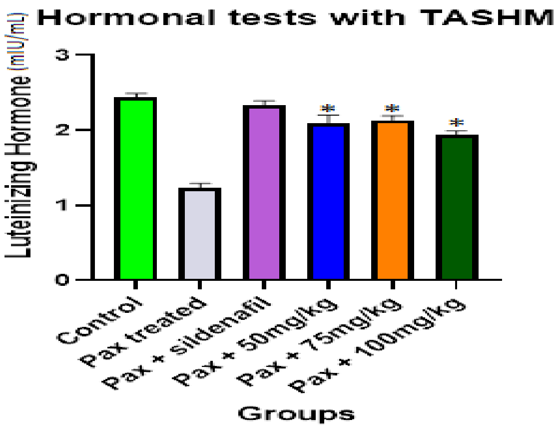

Figure 6: Variation in serum LH treated with TASHM

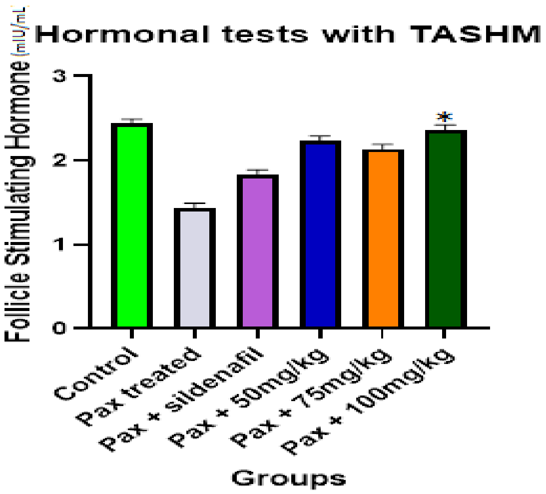

Fig. 7: Variation in serum FSH treated with TASHM

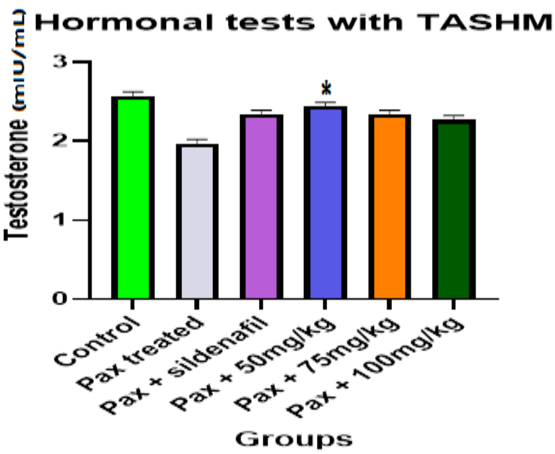

Fig.8: Variation in serum TH treated with TASHM

Histology report: Rats in the group that received 50 mg/kg of TASM showed normal histology, as well as well-preserved seminiferous tubules and spermatogenic elements as shown on Plate 2a, similar to the group treated with sildenafil citrate on Plate 1b. The paroxetine induced and treated with 75 mg/kg of TASM on Plate 2b, showed few intervening spaces within the interstitial and mild sloughing of the cells from the seminiferous epithelium. However, the group treated with 100 mg/kg of TASM on Plate 2c, exhibited a widespread degeneration of spermatogenic cells into the tubule lumen with severe impaired spermatogenesis.

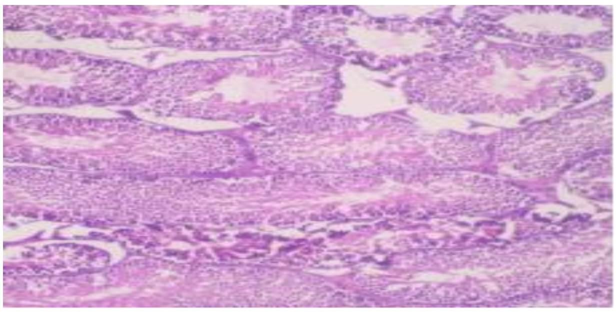

Plate 1a: Positive control group, induced with paroxetine but not treated.

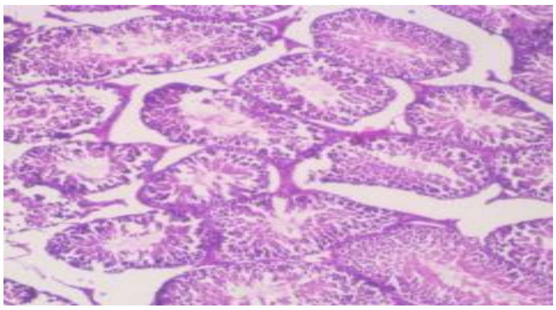

Plate 1b: Standard group, induced with paroxetine and sildenafil citrate.

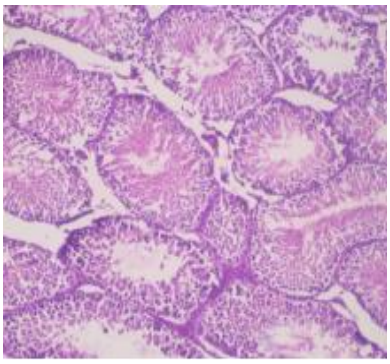

Plate 2a: Group induced with paroxetine and treated with 50 mg/kg of TASM

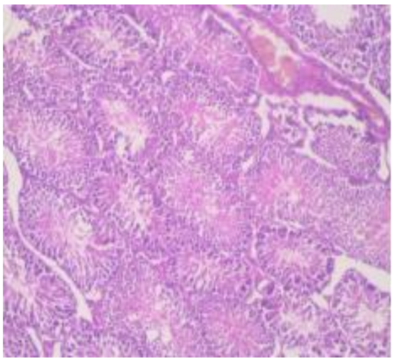

Plate 2b: Group induced with paroxetine and treated with 75 mg/kg of TASM

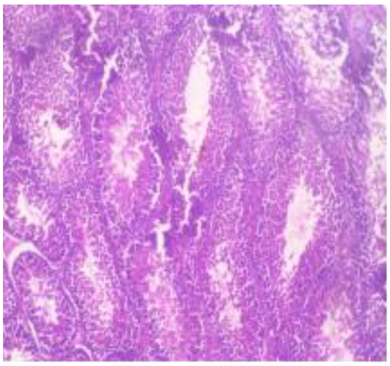

Plate 2c: Group induced with paroxetine and treated with 100 mg/kg of TASM

Plates 3a – 3c show that all groups treated with paroxetine at concentrations 50 mg/kg to 100 mg/kg of TASHM, presented with perfect histology, well-organized collection of cells and increased spermatogenesis. However, there were hypertrophic interstitial Leydig cells with a thickened basement membrane in the group with 75 mg/kg of TASHM. The group treated with 100 mg/kg of TASHM displayed loss of lumen architecture and spermatogenesis.

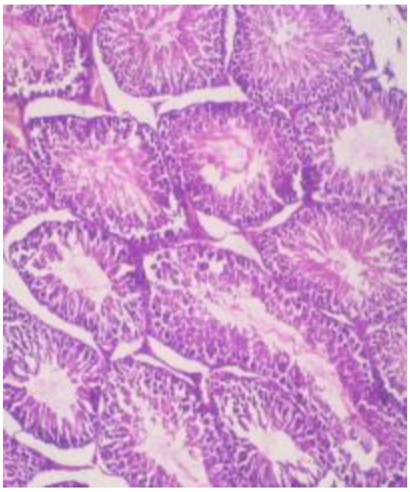

Plate 3a: Group induced with paroxetine and treated with 50 mg/kg of TASHM.

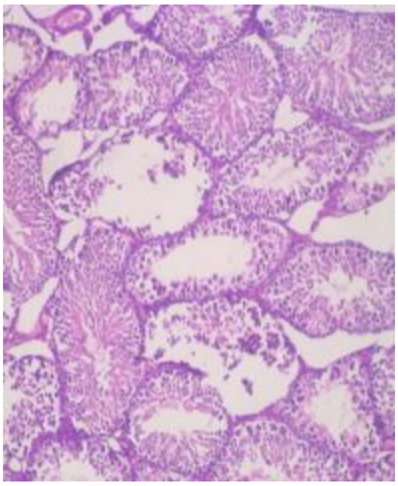

Plate 3b: Group induced with paroxetine and treated with 75 mg/kg of TASHM.

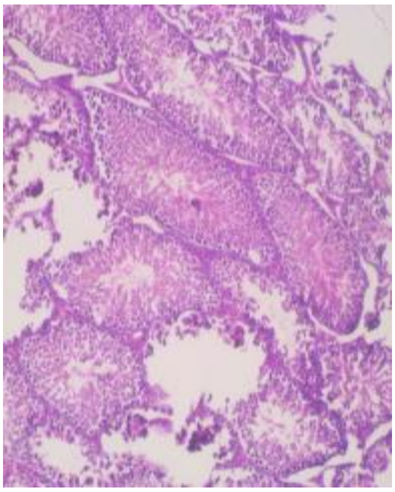

Plate 3c: Group induced with paroxetine and treated with 100 mg/kg of TASHM.

Discussion

The seeds of *H. umbellata*, roots of *T. avicennioides*, and *T. superba* were found to be rich in some secondary metabolites known to enhance erectile function in men. These metabolites included; alkaloids, saponins, tannins, phenolics, reducing sugar, cardenolides, cardiac glycosides, steriods, triterpenes, flavoniods. This report corroborates with the research conducted by Adeneye *et al*., (2019), which confirmed *H. umbellata* male fertility-enhancing components. The presence of libilov earlier detected in saponins, was found to be responsible for increase in men's sexual erection. Also protodioscin, which increases the level of dehydroepiandrosterone (DHEA) in infertile men (Arsyad, 1996). The mechanism of action is by increasing the conversion of testosterone into the potent dehydro-testosterone, as well as stimulating an increase in the sex drive, production of red cells, and muscle development (Arsyad, 1996). This process in turn contributes to the improvement of blood circulation, thereby increasing erection. Other phytochemical components of the plants such as tannins reduce the mutagenic activity of several mutagens, capable of inducing oxidative stress. The anti-oxidative property of tannins is important in protecting cellular oxidative damage, which helps in the inhibition of superoxide radicals (Petroski and Minich, 2020). Though this study did not conduct GC-MS, enough evidence abound in literature on the presence of some bioactive phytoconstituents found in *T. avicennioides* such as Tetratetracontane, L-Octadecene, Sulfurous acid, and Octacosame (Sulaiman *et al*., 2017). The seeds of *H. umbellata* also contain constituents such as 2-2/-Benzylidenebis (3 methylbenzofuran), Benzenemethanol, Pheno,6-methoxy-2[2-(2-quinoly) etheny, 2-Ethylacridine, Glibenclamide, 11-Octadecenoic acid, Urs-12-en-24-oic acid and 9-Octadecenoic acid (2) (Ladokun *et al*., 2018). Diabetes has been indicated as one of the causes of ED (Bruening *et al*., 2019), therefore the presence of 2-2/-Benzylidenebis (3 methylbenzofuran) in *H. umbellata* as an anti-diabetic compound, found in a reasonable quantity, will control blood sugar level and thus, increase penile erection. Therefore, the preponderance of these compounds may have contributed to the significant increase in erectile function, as evidenced by this current study.

NO is a principal intermediate of penile erection (Diaber *et al*., 2019) and a signaling molecule (Bankole *et al*., 2011). This has been demonstrated as a significant increase was observed in the level of NO in the treated groups of TASM and TASHM. It has been reported that neural transmitters such as acetylcholine, dopamine, and nerve cells are released from the cavernosal nerve endings aided by L-arginine during sexual arousal (Diaber *et al*., 2019). The L-arginine causes the stimulation of an enzyme nitric oxide synthase (NOS). To speed up the rate of reaction, the NOS further stimulates another co-factor enzyme known as Tetrahydrobiopterin (BH4). BH4 is a domain of (B cell lymphoma 2) Bcl-2 proteins that "plays a crucial role in regulating apoptosis" (Liu *et al*., 2016). The enzyme NOS and its cofactor (BH4) then catalyzes the release of NO from the endothelium (Diaber *et al*., 2019). NO stimulates the production of guanylyl cyclase which converts Guanosine Triphosphate (GTP) to cyclic Guanosine Monophosphate (cGMP) by removing one phosphate molecule. This process constantly increases the level of cGMP causing the conversion of Adenosine Triphosphate (ATP) to Adenosine Diphosphate (ADP), thereby stimulating cGMP-specific kinase to decrease the level of calcium ion thus, increasing potassium ion. The process will eventually cause the smooth muscle to relax, consequently would lead to an inflow of blood in turn resulting in an erection (Onal *et al*., 2016). Though the level of cGMP was not measured in this study, the evidence of significant increases in levels of NO at 75mg/kg in TASM and TASHM supports the possible increase in the level of cGMP and inflow of blood to enhance erection.

Sildenafil (Viagra), Vardenafil, Tadalafil, Udenafil, among others are synthetic drugs that have been identified as phosphodiesterase type 5 inhibitors (PDE5Is), which are antioxidants (Cai *et al*., 2020). These antioxidants increase sperm motility and sperm count in infertile men while reducing oxidative stress and increasing erectile function (Walczak-Jedrzejowska *et al*., 2013; Singh *et al*., 2016; Chen *et al*., 2019). The NO concentration of TASM at the doses of 50 mg/kg, 75 mg/kg, and 100 mg/kg had a significant increase than the group treated with sildenafil. TASM is suspected to have bonded to the active site of phosphodiesterase type 5, thus preventing it from reconverting the cGMP produced to Guanosine Monophosphate (GMP), thereby sustaining erection for a longer period as previously observed by Chen *et al*., (2019).

An increased level of testosterone has an enhanced increase in the level of sexual desire, arousal ability, sexual fantasies, and penile rigidity (Holka-Pokorska *et al*., 2014; Mahmoud and Ramadhan, 2021). With this, it could be ascertained that the higher testosterone content of the group treated with TASM at a concentration of 100 mg/kg may be one of the contributing factors responsible for the restoration and increase in sexual function, in the paroxetine-induced sexual dysfunction of male rats. The data generated from this research showed that the levels of TH in TASM were significant in the groups treated with 50 mg/kg, 75 mg/kg, and 100 mg/kg, while fluctuations in the levels were observed in other groups. It was also observed that groups treated with different concentrations of TASHM had a higher concentration of TH. More also, a significant increase in the level of LH was recorded in groups treated with 50 mg/kg of TASM, while FSH had its level significant increase at 100 mg/kg of TASHM. Thus, this report validates the work on the efficacy of plant extracts on testosterone concentrations (Smith *et al*., 2021).

LH and FSH are normally produced by the anterior pituitary called gonadotrophs and are necessary for the increase in the level of testosterone and the stimulation of gonads in males. LH helps in the release of steroids which correlates to the organic compounds identified in the plant extracts used.

Normal seminiferous tubules are necessary for the increased level of Follicle Stimulating hormone, which in turn leads to the increase in the level of adenosine mono phosphate (AMP) (Ulloa-Aguirre *et al*., 2018). It is interesting to note that the histological examination of the testes revealed that, the group treated with 50 mg/kg of TASM and TASHM showed normal histology, well preserved seminiferous tubules, spermatogenic elements, well-organized collection of cells, and increased rate of spermatogenesis. This also correlates with the results obtained in hormonal tests with TASM and TASHM, where high levels of follicle stimulating hormone were observed. The effects of luteinizing hormone on spermatogenesis are due to the stimulation of androgen production by the interstitial cells of the testis. This suggested that the high level of luteinizing hormone obtained in the group treated with 50 mg/kg TASM was due to the highly preserved interstitial cells by the plant extracts used.

Leydig cells have been explained to be responsible for high androgen production (Zirkin and Papadopoulos, 2018). The level of testosterone also depends on the number of Lydig cells in the stem cells. The hypertrophic interstitial Leydig cells with a thickened basement membrane were discovered in this study and these cells are known to produce testosterone in the presence of the luteinizing hormone. Luteinizing hormone secreted by the pituitary gland in response to gonadotrophin-releasing hormone (GnRH) from the hypothalamus, initiates steroid formation by binding to the leydig cell LH receptor (LHR), which through coupling to G-protein stimulates leydig cell cyclic adenosine 3/5/ - monophosphate (cAMP) production (Oyeleye *et al*., 2020). Consequently, these results indicated that both TASM and TASHM at 50mg/kg presented the best modulatory effect on the tissues as revealed by histology reports.

Conclusion

The results obtained from this study suggest that the combination of ethanol root extracts of *T. avicennioides*, *T. superba* and *H. umbellata* at 50 mg/kg, 75 mg/kg, and 100 mg/kg body weights, was very effective in reducing the damage done by paroxetine in male Wistar rats, with the dose of 75 mg/kg being the most effective in reducing the damage to the rats. The enhanced level of testosterone and nitric oxide-based mechanism may have supported an increase in penile erection and therefore not only validates their use by traditional medicine practitioners in the treatment of sexual dysfunction but also portrays these plants as good candidates in the treatment of erectile dysfunction. Consequently, the prospects of their involvement in drug development are imperative. The combination of these three plants has revealed the greatest quality in both the secondary metabolites and micronutrients apparent in the alleviation of erectile dysfunction in rats at 50 mg/kg in both TASM and TASHM mixtures. Finally, clinical studies of the bioactive principles and side effects evaluation of the roots of *T. avicennioides*, *T. superba* and seeds of *H. umbellata* are important areas for further research.

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