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Original Article

Spondias mombin LEAVES ATTENUATE SOME BIOMARKERS OF ERECTILE AND CARDIOVASCULAR DYSFUNCTION INDUCED BY PAROXETINE HYDROCHLORIDE IN RATS

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ABSTRACT

Spondias mombin leaf is a medicinal plant used in folk medicine to increase male sexual function, however, studies providing scientific proof are scarce. Therefore, this study examined the regulatory influence of *Spondias mombin* leaf ethanol extract (EESML) parameters of erectile activity in male Wistar rats. Of the thirty rats used, five of them, naïve control, as (Group A) was given distilled water while the twenty-five were first administered paroxetine orally for 21 days. After this period, they were reorganized. For seven days, the following treatments were administered to five groups of five rats each: group B received distilled water; group C received 4 mg/kg sildenafil citrate; group D received 15 mg/kg EESML; group E received 30 mg/kg EESML; and group F received 45 mg/kg EESML. On the eighth day, all animals were sacrificed under dichloromethane anesthesia. The study then assessed the inhibitory potential of EESML on Phosphodiesterase-5 (PDE-5), Nitric oxide (NO) and arginase activity in penile and cardiac homogenates. The concentration of Troponin I and lipid profile were assessed specifically in serum as well as 17β-, 3β- hydroxysteroid dehydrogenases and testosterone concentration increased in parallel with the significant ($p < 0.05$) inhibition of PDE-5 and arginase by EESML in both tissues, which was more pronounced at higher doses. During comparison with untreated and naïve controls, the specific activities of 17β, 3β hydroxysteroid dehydrogenase, and testosterone levels were considerably higher ($p<0.05$) in all EESML-treated groups. Inversely, EESML significantly decreased the amount of Troponin I in the serum. Similar trend was noticed in the serum concentration of lipids except for HDL-C which increased following treatment with EESML. EESML may be effective in reversing erectile dysfunction, as evidenced by its favorable comparison with it regulatory activity in rats treated with sildenafil citrate and naïve control, particularly in the group in which 45 mg/kg body weight of EESML was administered.

KEYWORDS: EESML, Phosphodiesterase-5, Arginase, 17β- and 3β- hydroxysteroid dehydrogenase, Testosterone, Troponin I.

1. INTRODUCTION

Sexual function is an important behavioral characteristic necessary for reproduction. Erectile dysfunction (ED) is a dysfunction that affects sexual function and has a great negative effect on couples. In Nigeria, it is one of the challenging disorders (Akinjiyan *et al*., 2022). A man is diagnosed with ED when he fails to keep up a strong enough erection to satisfy sexual pleasure (Akbari *et al*., 2020; Eserdag *et a*l., 2021).

The risk factors of penile erection disorder can originate from peripheral causes, such as injury to the smooth muscles or fibrous tissues, nerves, and arteries (Akorede *et al*., 2024). It may also result from psychological issues like anxiety, stress, depression, guilt, and fear of sexual failure (Saadi *et al*., 2024). Additionally, ED can be associated with medical conditions such as diabetes, kidney disease, vascular disorders, and neurological illnesses, as well as adverse effects of certain medications, including antihypertensives, antihistamines, tranquilizers, antidepressants, cimetidine, and appetite suppressants. Hormonal imbalances, particularly low testosterone concentration, can also play a role in ED. Often, the condition arises from a combination of these factors (Muritala, 2018).

The risk factors and cardiovascular disorders are closely associated with erectile dysfunction (Mostafaei *et al*., 2021). Consequently, men with a history of erection difficulties are highly vulnerable to dying from cardiovascular dysfunction (Terentes-Printzios *et al*., 2022). ED and cardiovascular disease are caused by similar factors, including advanced age, hypertension, diabetes, insulin resistance, tobacco use, increased body mass index (BMI), higher cholesterol levels, and reduced concentrations of good cholesterol (high-density lipoprotein) (Mostafaei *et al*., 2021). Studies have linked ED with smooth muscle and endothelial cell failure (Theofilis *et al*., 2021; Argiolas *et al*., 2023). Theofilis *et al*. (2021) reported that ED patients, regardless of cardiovascular disease status, exhibited elevated levels of inflammatory and endothelial prothrombotic

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markers/mediators. The findings highlighted that the increased levels underscore the importance of dysfunction in endothelial cells in the development of ED in connection with cardiovascular disease.

Pharmacological treatments of ED comprise dopamine receptor inhibitors, beta-adrenergic receptor inhibitors, and sildenafil citrate. Due to the adverse effects of these medications, which include priapism, flushing, sight problems, hypotension, diarrhea, and dyspepsia, it is necessary to investigate alternative sources (Malviya *et al*., 2016). Therefore, continued study of medicinal plants with aphrodisiac potential is essential, as a cost-effective and safe alternative therapy. *Spondias mombin* Linn (*S. mombin*) is traditionally used in Southwestern Nigeria in traditional medicine as an aphrodisiac. It is also indicated for the management of mental health disorders, regulating diabetes mellitus, and enhancing and preserving memory (Oloye *et al*., 2017; Boadu *et al*., 2022). The aim of this study was to investigate the impact of ethanolic *Spondias mombin* leaves extract (EESML) on some markers of erectile function in male Wistar rats.

Material And Methods:

Materials:

Collection and identification of plant samples:

Fresh *Spondias mombin* leaves were obtained from an herb seller at the Oja Oba market in Ilorin, Kwara State. Identification was done at the herbarium unit of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, where a voucher number UILH/001/1148/2024 was assigned.

Reagents, Chemicals, and Assay Kits:

Ethanol (analytical grade) (Lot No: 20210315), Dichloromethyl, Paroxetine hydrochloride (Milpharm), Sildenafil citrate (Viagra) (Lot no.:A471719, a product of Pfizer, New York), Tween-80 (a product of BDH laboratory chemicals, Poole, England), normal saline (Dana pharmaceuticals), L- arginine, cyclic guanosine monophosphate (Lot no.: BCBJ1515V), The Calbiotech, Inc assay kit for testosterone (Catalog No. TE373S); Assay kits for total cholesterol, triglyceride, low density lipoprotein cholesterol, high density lipoprotein cholesterol, Assay kit for 17 β- HSD and 3 β-HSD. Trizma base (Lot no.: BCBJ8442V), Sigma Aldrich Chemical Company, located in St. Louis, USA, produced Crotalus adamanteus venom (SLBR1148V); Santa Cruz Biotechnology, located in Dallas, Texas, produced imidazole (lot number: B0212); and the assay, kit for cardiac Troponin I (human cTn- I/TNNI3 CLIA kit) (Catalog No: E-CL-HO495) was a product of Elabscience United State of America.

Experimental Animals:

Thirty adults male Wistar rats weighing between 150 and 220 grams were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were acclimatized for three weeks and were kept at room temperature in the laboratory, fed with rat chow and water. The U.S. National Academy of Sciences issued guidelines for the use and care of experimental animals, which served as the basis for their treatment.

2. METHODS

Extract Preparation:

The leaves of *Spondias mombin* were gently washed under running tap water to remove dirt and completely dried at room temperature. The dried plants were pulverized in an electric blender in preparation for extraction. For extract preparation, 400 g of the powdered plant material was placed in a glass jar and macerated in 750 mL of ethanol for 48 hours under continuous stirring. The homogeneous sample was filtered through Whatman No.1 filter paper, and the filtrate was concentrated in a water bath at 40 °C. The resulting sample was stored in an air-tight container at 4°C. The extract percentage was 30%. The doses of EESM used in this study were reconstructed from the stock extract daily as required.

High-Performance Chromatography (HPLC) Evaluation:

Utilizing High-Performance Liquid Chromatography (HPLC) and conventional methods, the phytochemical profiling of *Spondias mombin* leaf extract was investigated (Snyder *et al*., 2011; Dong, 2013). Briefly, 20 mL of acetonitrile and 10 g of powdered leaf material were extracted in a separating funnel, shaken vigorously for 20 minutes, and the filtrate was brought up to 25 mL. Each run involved injecting 5µL of the sample into a Shimadzu Nexera MX HPLC system that was equipped with Diode Array Detector and a Bondapak C18 column (100 mm × 4.6 mm, 7 µm). (DAD) set at 254 nm. Compounds were identified by comparing their retention times with reference standards using High-Performance Liquid Chromatography (HPLC) and conventional methods. The phytochemical profiling of *Spondias mombin* leaf extract was investigated (Snyder *et al*., 2011; Dong, 2013), and quantification was performed by relating sample peak areas to those of the reference standards.

$$\text{Sample concentration} = \frac{Sample\ peak\ area \times Standard\ concentration}{\text{Peak area of Standard}}$$

***In Vivo* Experimental Studies:**

Induction of Erectile Dysfunction:

Thirty male rats were induced with erectile dysfunction by oral administration of 10 mg/kg body weight of paroxetine suspension, which was prepared daily in Tween-80 and normal saline for 21 days using an oropharyngeal cannula. Five non- erectile dysfunction rats received only normal saline solution and served as naïve control (Malviya *et al*., 2011; Muritala *et al*., 2025)

Experimental Design and Care of Rats:

Thirty (30) male Wistar rats (15 weeks old, 180–200 g) from the animal holding unit of the department of biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were given unlimited access to water and a commercial meal and were acclimatized for two weeks. The institution's ethical committee and the US National Institutes of Health (NIH) ethical criteria were applied when working with experimental rats. The rats were housed in stainless steel cages under a 12-hour dimly lit cycle in a room kept between 25 and 28 degrees Celsius during the experiment.

Following two weeks of acclimatization, paroxetine suspension was administered orally at a dose of 10 mg/kg for 21 days to 30 male rats to induce erectile dysfunction. Nitric oxide depletion and increased phosphodiesterase-5 activities in tissues were used to validate erectile dysfunction. Paroxetine suspension was prepared daily in Tween-80 and saline solution. To provide a baseline for comparison, the non-induced control rats were given 0.75 mL of normal saline. The untreated group comprised rats that were induced but received distilled water, providing a measure of paroxetine's standalone effect. Positive control consisted of rats that were induced and then treated with 4 mg/kg body weight of sildenafil citrate. After this period, the remaining three groups received 15, 30, and 45 mg/kg B. wt. of *Spondias mombin* ethanolic extract (EESML): 15 mg/kg, 30 mg/kg, and 45 mg/kg. These groups were named Paroxetine + 15 mg/kg EESML, Paroxetine + 30 mg/kg EESML, and Paroxetine + 45 mg/kg EESML. Although the amount of paroxetine was recommended by Ademosun *et al*. (2019), the dose of *Spondias mombin* extract was chosen based on earlier research by Gobinath *et al*. (2022). The experiment was carried out for

twenty-eight days. Rats under dichloromethane anesthesia were killed on the final day. After being removed for biochemical analysis, the heart and penile tissues were ground up in a Teflon glass tissue grinder and centrifuged. The supernatant was stored at -20 °C in preparation for further testing.

Erectile Function Assays:

Activity of Phosphodiesterase-5 (PDE-5) Enzyme:

The dialyzed fraction of the extract containing the enzyme was suspended in a reaction mixture containing 0.36 µmoL of cyclic guanosine monophosphate (cGMP), 1.8 µmoL of $MgSO_4$ and 36 µmoL of Tris buffer, pH 7.5, with a suitable dilution of the phosphodiesterase sample being tested in a total volume of 0.9 mL. This mixture was incubated at 37 ºC for 30 minutes. After the first 20 minutes of incubation, 0.1 ml of a Crotalus adamanteus venom solution, containing 0.1 mg of venom in 1x 10^{-2} M Tris, pH 7.5. The reaction was terminated by the addition of 0.1 mL of ice-cold 55% trichloroacetic acid (TCA). After addition of TCA, the solution was centrifuged at 2000 rpm for 10 minutes, the precipitate was removed, and 500 µL of the supernatant fluids were analysed for inorganic phosphate as described by Fiske and Subbarow (1925), modified by Butcher and Sutherland (1962), with a slight modification as described below:

The colour was developed by the addition of 1mL of reagent C consisting of 9% ascorbic acid (solution B) and ammonium molybdate (solution A) in a 4:1 ratio which was prepared just before use) and read at 820 nm on a spectrophotometer. The amount of inorganic phosphate released per unit time was extrapolated from the calibration curve using 1 mM disodium hydrogen orthophosphate as a standard.

Nitric Oxide:

Nitric oxide levels in tissues were determined using the Griess reagent following the method described by Green *et al*. (1982). Tissue homogenate was deproteinized by treating 500 µL of appropriately diluted homogenate with 100 µL of 5% $ZnSO_4$ and 100 µl of 0.3 M NaOH. The reaction mixture was then centrifuged at 4000 g for 20 minutes. Thereafter, 100 µl of the supernatant was treated with 1000 µL of Griess reagent, and the absorbance was measured at 540 nm. Griess reagent was used as a blank. The nitrite concentration was determined using a calibration curve for sodium nitrite.

Arginase activity:

Urea production was measured using Ehrlich's reagent, with tissue homogenate supernatant as the enzyme source. The reaction mixture (1.0 mL) contained a 50nM enzyme preparation. For 10 minutes, 0.1 M arginine and 1.0 mM $MnCl_2$ were incubated at 37 °C in 0.1 mM Tris-HCl buffer (pH 9.5). The percentage inhibition of arginase inhibitory activity was computed when the reaction was stopped using the urea assay kit.

Assay for Hydroxysteroid Dehydrogenase Enzyme:

The Marcus and Talalay (1956) method was adopted. The increase in absorbance at 340 nm, which represented Nicotinamide adenine dinucleotide-reduction when androsterone or testosterone were used as substrates under certain assay conditions, was used to calculate reaction velocity. One mg/ml of pure enzyme was dissolved in Tris-HCl buffer (0.03 M, pH 7.2) with EDTA (0.001 M). One enzyme unit was defined as the amount that catalyzes the conversion of 1 µmol of NAD per minute at 25 °C and pH 9.0. For further dilution, the buffer was utilized. The enzyme was isolated from cell lysates by sonicating a 50 mg/ml cell solution in the same Tris-HCl buffer and centrifuging the resultant clear supernatant. A rate of 0.02-0.04 ΔA/min was obtained by diluting the isolated enzyme.

At 25°C, the spectrophotometer was calibrated to 340 nm. 0.6 ml of 0.166 M sodium pyrophosphate was added to each cuvette, followed by 0.6 ml of a solution of 0.166 M sodiumpyrophosphate, 0.2 ml of 0.0043 M NAD, 2 ml of reagent-grade water, and 0.1 ml of the enzyme preparation. For the 17β-hydrosteroid dehydrogenase assay, 0.1 ml of testosterone solution was introduced after the spectrophotometer was incubated for 3–4 minutes to achieve a consistent temperature and reference rate. ΔA340/min was computed from the initial linear phase after absorbance was measured at 340 nm for three to four minutes. The 3β-hydrosteroid dehydrogenase assay followed the same procedure, and androsterone served as substrate.

Calculation

$$\text{Units/mg} = \frac{\text{A340/min}}{\text{6.22 x mg enzyme/ml reaction mixture}}$$

Serum Testosterone Concentration:

The serum testosterone concentration was quantitatively determined using the direct human testosterone enzyme immunoassay (EIA) kit, following the method described by Tietz (1995).

Before proceeding with the assay, all reagents were brought to room temperature (20-25 °C). 50 µL of the standards, control, or specimen were added to the assigned well. 100 µL of working testosterone-enzyme conjugate reagent was added to each well. 50 µL Biotin reagent was added to each well. The microplate was gently swirled for 20-30 seconds to mix the reagents. The plate was covered and incubated at room temperature for 60 minutes. The liquid was removed from the wells and washed three times with 300 µL of 1X wash buffer. 100 µL of TMB substrate was added to each well. The plate was covered and incubated at room temperature for 30 minutes. 50 µL stop solution was added into each well and gently mixed for 15-20 seconds. The absorbance was read on an ELISA reader for each well at 450 nm, within 15 minutes after adding the stop solution.

Protein Concentration Determination:

Rat body tissues and serum total protein levels were determined using the Gornall *et al*. (1949) method. For each test, 1.0 mL of sample and 4.0 mL of Biuret reagent were mixed simultaneously, thoroughly swirled, and allowed to stand at room temperature for 30 minutes to allow color development. One milliliter of pure water was used as a blank when measuring the absorbance at 540 nm. The protein concentration from the egg-albumin calibration curve was calculated using the formula below:

$$\text{Protein concentration (mg/ml)} = Cs \times F$$

where F is the dilution factor, and Cs is the concentration derived from the calibration curve.

The protocol used to generate the protein calibration curve is described below:

A stock solution of egg albumin (10 mg/mL) was prepared as the protein standard. A well-cleaned test tube was filled with aliquots of 0.0 to 1.0 mL of the stock solution. They were subsequently adjusted to a final volume of 1 ml using distilled water. After that, four milliliters of biuret reagent were added to each tube, for a total of five milliliters. The absorbance of the mixtures was measured at 540 nm after 30 minutes of standing at room temperature. To calculate the protein concentration, each absorbance value was divided by 0.0684.

Determination of lipid profile:

Determination Total Cholesterol Concentration:

The total cholesterol concentration was determined by the colorimetric method as described by Fredrickson *et al*.

(1967). Twenty microliters of a sufficiently diluted sample, standard, or distilled water was dispensed into test tubes labeled with the relevant designation using a miniature pipette. Each tube was then filled with 2000 µl of the reagent combination, which included phenol, 4-aminoantipyrine, sterol oxidase, peroxide, cholesterol cholesteryl ester hydrolase, and buffer (pH 6.8). After the reagents were fully mixed, the mixture was incubated at 37 °C for 5 minutes. Readings of absorbance for both the sample and the standard were taken at 546 nm, using the reference solution as the blank.

Cholesterol Concentration (mmol/L) was determined using the Equation:

$$\text{Cholesterol concentration} = \frac{(\text{Absorbance of sample} \times \text{Concentration of standards})}{\text{Absorbance of Standard}}$$

Where the standard concentration was 5.10 mmol/L

Determination of Triglyceride Concentration:

Serum triglyceride concentration was determined using the method described by Hainline *et al*. (1980). Sterile test tubes with the proper labels were filled with 10 microliters of distilled water, standard, and adequately diluted sample. Each tube received 100 µL of a reagent that included 4-aminophenazone, glycerokinase, ATP, glycerol phosphate oxidase, lipases, and peroxidase. Following mixing, the mixture was allowed to stand at ambient temperature (20–25 °C) for 10 minutes. Every sixty minutes, the absorbance of the standard and sample was measured at 500 nm relative to the blank.

Concentration of Triglyceride (mmol/L) was Determined Using the Equation:

$$\text{Triglyceride concentration} = \frac{\text{Absorbance of sample} \times \text{Concentration of standards})}{\text{Absorbance of Standard}}$$

Where the standard concentration was 2.21 mmol/L

Determination of High-Density Lipoprotein (HDL) Cholesterol:

Plasma high-density lipoprotein cholesterol (HDL-C) concentration was determined using the method described by Albers *et al*. (1978). Clean, well-labeled test tubes were filled with 200 µL of properly diluted sample, standard, and distilled water. Each test tube was then filled with 500 µL of a reagent that contained magnesium chloride and phosphotungstic acid. After a gentle vortex, the resulting solution was heated at room temperature for 10 minutes. Afterward, the tubes were centrifuged for 10 minutes at 4000 rpm. The resulting transparent upper layer was collected within two hours, and the CHOD-PAP method described earlier was used to determine the cholesterol content.

CALCULATION

HDL cholesterol concentration (mmol/L) was calculated using the following formula:

$$\text{HDL cholesterol concentration (mmol/L)} = \frac{\text{A sample} \times \text{C standard}}{\text{A standard}}$$

C standard = 5.10 mmol/L

Determination of Low-Density Lipoprotein Cholesterol (LDL)

Serum low-density lipoprotein cholesterol (LDL-C) concentration was calculated using the Friedewald *et al*. (1972) method. The equation below was used to assess LDL-C.

LDL Cholesterol (mmol/l) = Cholesterol – VLDL- HDL

$$\text{VLDL} - \text{C} = \frac{\text{TAG}}{2.2}$$

Atherogenic Index:

The Arteriosclerotic Index was computed using the method described by Niroumand *et al.* (2015). The computation was done using the expression:

Log_{10} (TG/HDL-C).

Determination of Troponin Concentration:

In the first two columns, 100 µL of the standard solution was dispensed to one well each and duplicated in the second row side by side. Similarly, 100 µL of each sample was dispensed into the remaining wells in duplicate. The plate was sealed with the adhesive cover provided in the kit and incubated at 37 ℃ for 90 minutes. Cover provided in the kit and incubated for 90 minutes at 37 °C. All solutions were carefully dispensed to the bottom of the micro-CLIA well, preventing contact with the inner walls and foam formation.

The wells were decanted without being cleaned after incubation. The biotinylated detection antibody solution (100 µL) was promptly added to each well. The plate was gently mixed, then sealed once more and placed in an incubator at 37 °C for 1 hour. Each well was loaded with 350 µL of wash buffer, allowed to soak for one to two minutes, and then separated after the contents were discarded. The dish was dried by turning it onto absorbent paper. After this washing step was repeated, 100 µL of the HRP-conjugate solution was added to each well. The plate was sealed again and then placed in an incubator at 37 °C for 30 minutes.

The wells were drained, and the cleansing procedure was repeated five times following the incubation period. The plate was heated after 100 µL of the substrate solution was added to each well, and a new plate sealer was applied at 37 °C for no more than 5 minutes, while the plate was shielded from light. Each well's relative light unit value was ascertained immediately.

Statistical Analysis:

All data were presented as means of five determinants ± standard error of mean (SEM). Statistical evaluation was performed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for multiple comparisons.

3. RESULTS

Bioactive Components Identified in the Ethanol Extract from the Leaves of *Spondias mombin*:

The bioactive component of *Spondias mombin* leaf ethanol extract was identified as 19 distinct phytochemical constituents (Figure 1 and Table 1) by HPLC, based on retention times, peak areas, and peak heights. Among these, quercetin exhibited the highest peak area and height (retention time 11.050 min), followed by kaempferol (12.166 min). Other notable compounds with moderate peaks included geraniol, mescaline, nicotine, berberine, and caffeic acid, while gallic acid, ursolic acid, chelerythrine, vincamine, psilocin, ellagic acid, citral, and morphine was present in lower abundance. No overlapping retention times were observed. The total peak area of 15,831.16 indicates the overall abundance of UV-absorbing phytochemicals in the extract.

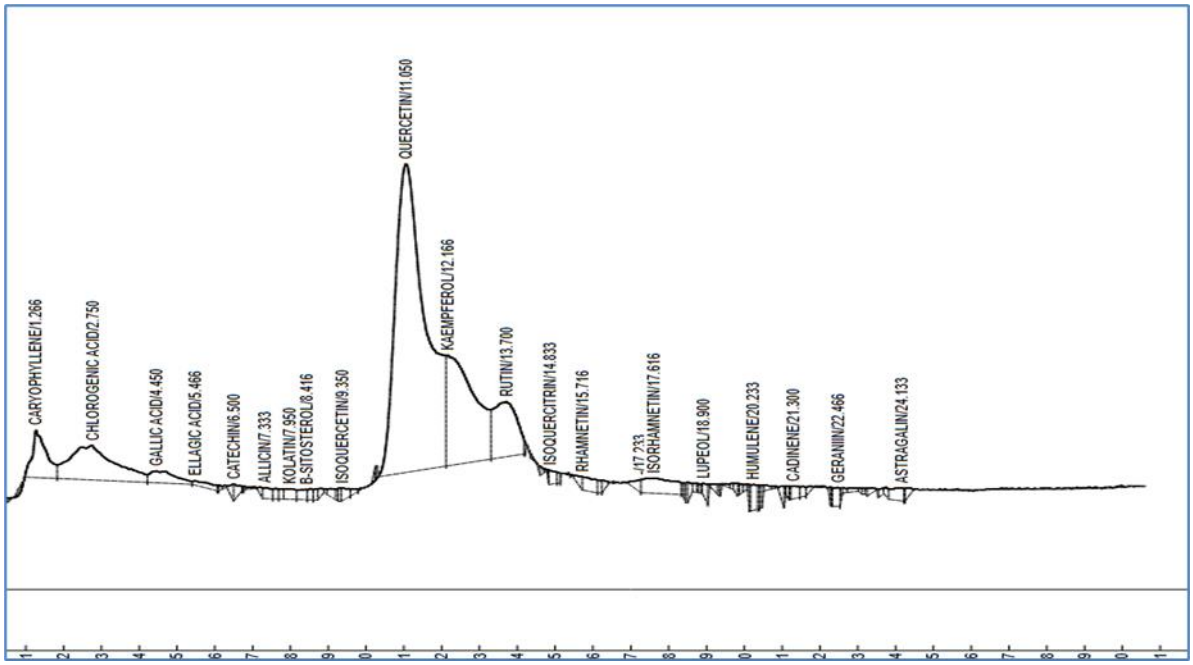


Figure 1: Identified Phytochemicals in *Spondias mombin* leaves using High Performance Liquid Chromatography (HPLC)

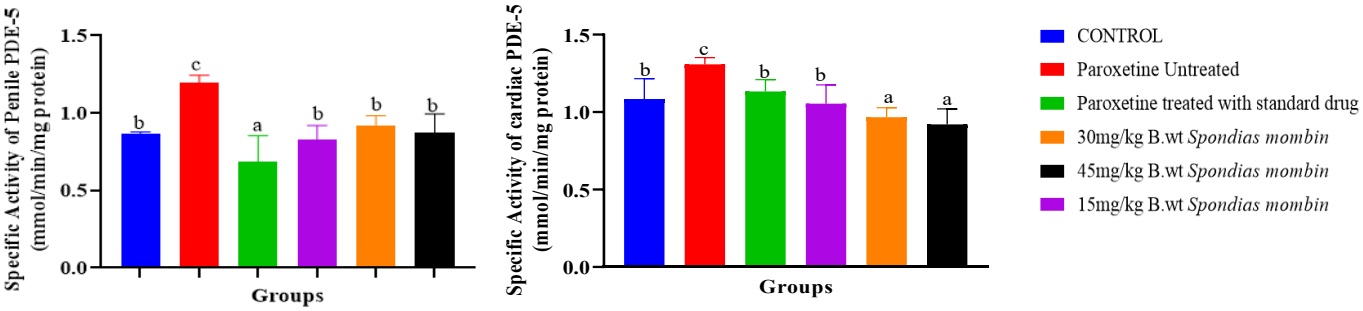
Table 1: Identified bioactive components in ***Spondias mombin leaves*** ethanol extract

No.	Compound Name	Retention Time (min)	Area	Height	External	Unit
1	Caryophyllene	1.266	610.2830	23.413	0.0000	
2	Chlorogenic acid	2.750	1422.0485	16.960	0.0000	%
3	Gallic acid	4.450	282.2360	5.778	28.2236	ppm
4	Ellagic acid	5.733	92.2670	2.116	9.2267	ppm
5	Catechin	6.483	92.2670	8.408	0.0000	
6	Allicin	7.333	81.9950	5.385	0.0000	
7	Kolatin	7.950	102.3055	5.087	0.0000	
8	B-Sitosterol	8.816	82.3060	5.048	0.0000	
9	Isoquercetin	9.350	69.3030	6.499	0.0000	
10	Quercetin	11.050	8328.9480	151.498	0.0000	
11	Kaempferol	12.166	2768.5260	54.264	0.0000	
12	Rutin	13.700	1163.9390	27.047	0.0000	
13	Isoquercitrin	14.833	81.3430	7.231	0.0000	
14	Rhamnetin	15.716	152.6880	6.464	0.0000	
15	Isorhamnetin	17.616	435.2880	7.511	0.0000	
16	Lupeol	18.900	65.2410	5.403	0.0000	
17	Humulene	20.233	136.4740	12.774	0.0000	
18	Cadinene	21.300	81.9340	6.247	0.0000	
19	Geraniin	22.466	109.0890	9.204	0.0000	
20	Astragalin	24.133	138.0130	6.256	0.0000	

Specific Activities of phosphodiesterase 5 in Penile and Cardiac organs of paroxetine Treated ED Male Rats After Oral administration of *Spondias mombin* leaf Extract in Ethanol:

The result of PDE-5's particular activity in the penile tissue of rats given paroxetine is shown in Figure 1A. The oral administration of ethanol leaf extract of *Spondias mombin* leaves (EESML) significantly ($P<0.05$) decreased PDE-5 specific activity in proportion to the dose in male Wistar rats; however, the reduction was not as great as in rats administered the usual medication. PDE-5 activity significantly decreased in rats given 15 mg/kg body weight of EESML, but did not significantly change in rats given 30 or 45 mg/kg. PDE-5 activity levels were similar to those found in the naïve control group across all EESML dosages.

The phosphodiesterase 5 (PDE-5) activity in the cardiac organ of Wistar rats given paroxetine is shown in Figure 1B. The ethanol extract of *Spondias mombin* leaves administered orally significantly (P<0.05) decreased PDE-5 specific activity in the hearts of male Wistar rats as the dosage increased. In contrast to rats in the naive control group and those given a conventional medicine at a dose of 4 mg/kg body weight, rats given 15 mg/kg body weight of this leaf extract exhibited positive specific activity of phosphodiesterase-5.



A: Penile PDE-5 B: Cardiac PDE-5

Figure 1: Specific activities of PDE-5 in penile (A), and cardiac (B) organ of paroxetine induced male rats following oral treatment with ethanol extract of *Spondias mombin* leaves

Nitric Oxide Concentrations in the Cardiac and penile Organs of paroxetine-induced erectile- dysfunction male Wistar Rats Following Oral Treatment with Ethanol *Spondias mombin* Leaf Extract:

As shown in Table 2, nitric oxide levels in the penile tissue were considerably lower (P<0.05) in the untreated rats compared with the naïve control group. However, EESML treatment resulted in a significant (P<0.05) rise in paroxetine-treated male Wistar rats' penile nitric oxide concentration.

Rats given various dosages of EESML had significantly (P < 0.05) higher nitric oxide concentrations than those in rats administered paroxetine. Treatment with sildenafil citrate showed a positive correlation with nitric oxide content in rats treated with various doses of the extract. However, compared with the level in the naive control, there was a notable variation in penile nitric oxide concentration among rats given varying doses of the extract.

The heart's nitric oxide content was notably lower (P<0.05) in the untreated and paroxetine-treated groups compared to the naive control and standard drug-treated groups in male Wistar rats given paroxetine. The concentration of nitric oxide in their cardiac organ increased significantly (P < 0.05) following administration of EESML in a dose-dependent manner. In this study, the concentration of nitric oxide in Wistar male rats administered 45 mg/kg body weight of EESML increased significantly (P < 0.05), and the findings showed a significant difference between the 30 and 45 mg/kg body weight EESML dosages.

On the contrary, rats given 30 mg/kg and 45 mg/kg EESML had higher nitric oxide concentrations than rats given a standard drug. Furthermore, compared to the naïve control, the extract impact at 45 mg/kg body weight was favorable.

Table 2: Concentration of penile and cardiac nitric oxide in paroxetine treated male rats after oral treatment with ethanol extract of *Spondias mombin* leaves

Groups	Concentration of penile nitric oxide (mg/ml)	Concentration of cardiac nitric oxide (mg/ml)
Control	77.78 ± 9.84^{c}	71.70 ± 3.24^{b}
Paroxetine Untreated	60.94 ± 2.68^{a}	61.87 ± 1.36^{a}
Paroxetine treated with a standard drug	74.17 ± 6.79^{b}	66.26 ± 1.77^{b}
15mg/kg B.wt *S. mombin*	71.88 ± 4.43^{b}	61.40 ± 1.98^{a}
30mg/kg B.wt *S. mombin*	71.63 ± 1.17^{b}	67.70 ± 6.51^{b}
45mg/kg B.wt *S. mombin*	67.22 ± 2.60^{b}	78.83 ± 2.00^{c}

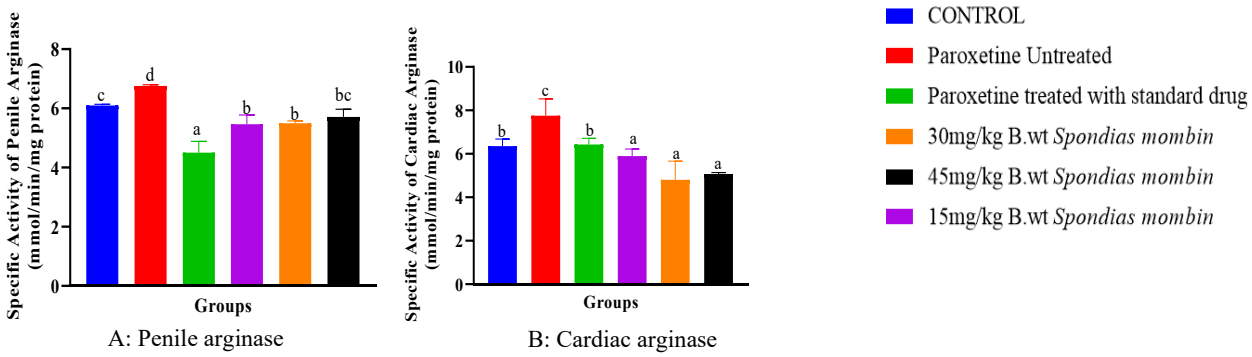
the naïve control group. While the usual medication did not result in a significant (P<0.05) drop in penile arginase activity, oral therapy with EESML provided a substantial (P<0.05), dose-dependent reduction.

Specific Arginase Activity in the Male Wistar Rats' Cardiac and penile Tissues After Administering paroxetine with Ethanol *Spondias mombin* Leaf Extract:

As illustrated in Figure 2A, paroxetine administration led to a marked elevation in penile arginase activity in comparison to

Nevertheless, rats given varying dosages of EESML showed no appreciable variation in arginase specific activity. Additionally, the specific activity of arginase in rats treated with these various dosages of EESML (15, 30, 45 mg/kg B.wt) showed a favorable comparison with that in rats in the same group.

Figure 2B shows the outcome of specific arginase activity in the cardiac organ of rats given paroxetine. Compared with the untreated rats, the specific activity of arginase increased significantly in the naive control. The oral treatment with EESML markedly ($P < 0.05$) reduced the specific activity of arginase in the heart in a dose-dependent manner. There was no discernible variation in the arginase specific activity of rats given different dosages of EESML. However, there was a substantial difference in arginase specific activity between rats in the naïve control group and those receiving the usual treatment. Furthermore, compared with rats in the naive control group, the specific activity of arginase in rats administered a standard medicine was higher.



A: Penile arginase B: Cardiac arginase

Figure 2: Arginase specific Activity within the penile (A) and heart (B) tissues of male rats treated paroxetine followed by administration of ethanol extract of *Spondias mombin* leaf. Data represent the mean ± SEM of five measurements. Bars within the same group with different superscripts differ significantly from one another ($P < 0.05$).

Serum 3β-Hydroxysteroid Dehydrogenase (3β-HSD) and 17β-HSD Specific Enzyme Activities in paroxetine-Administered Male Wistar Rats Followed by Oral Treatment with Ethanol Extract of *Spondias mombin* Leaf:

Table 3 shows serum 3β- and 17β- hydroxysteroid dehydrogenase specific enzyme activities in paroxetine - administered male rats following treatment with ethanol extract *S. mombin* leaf. Untreated rats exhibited a significant decrease in both enzyme activities ($P < 0.05$). Oral treatment with ethanol extract of *Spondias mombin* leaf significantly ($P < 0.05$) increased the activities of both enzymes compared to the untreated paroxetine group. The specific activities of 3β- and 17β- hydroxysteroid dehydrogenase of the group of rats that received 45 mg/kg body weight EESML were comparable to the group of animals that received sildenafil citrate. There was no significant ($P < 0.05$) difference in serum 3β- hydroxysteroid dehydrogenase specific enzyme activities of rats that received different doses of EESML and the control group.

Table 3: Serum 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-HSD Specific enzyme activities in paroxetine- administered male Wistar rats followed by oral treatment with ethanol extract of *Spondias mombin* leaf.

Groups	Specific activities of 3β- HSD (mmol/min/mg protein)	Specific activities of 17β- HSD (mmol/min/mg protein)
Control	0.31 ± 0.03^{c}	0.36 ± 0.04^{c}
Paroxetine Untreated	0.15 ± 0.03^{a}	0.19 ± 0.03^{a}
Paroxetine treated with a standard drug	0.28 ± 0.04^{b}	0.36 ± 0.02^{c}
15mg/kg B.wt *Spondias mombin*	0.33 ± 0.04^{c}	0.29 ± 0.05^{b}
30mg/kg B.wt *Spondias mombin*	0.32 ± 0.02^{c}	0.35 ± 0.04^{c}
45mg/kg B.wt *Spondias mombin*	0.29 ± 0.03^{b}	0.32 ± 0.04^{c}

Data represent the mean ± SEM of five measurements. Bars within the same group that have different superscripts are significantly different from each other ($P < 0.05$)

Serum Testosterone Concentration in paroxetine – Administered Male Wistar Rats Followed by Oral Treatment with Ethanol Extract of *Spondias mombin* leaf:

The serum testosterone concentration of rats given paroxetine orally is shown in Figure 3. Testosterone levels in the untreated rats were significantly lower than those in the naive control group ($P<0.05$). Testosterone concentration in rats administered EESML for seven days post-administration of paroxetine significantly ($P<0.05$) increased when compared with both the untreated group and the group that received sildenafil citrate. This increase was significantly ($P<0.05$) different from the naïve control group. Rats administered 30 mg/kg body weight of the extract showed the highest increase in testosterone concentration, followed by those administered 15 mg/kg, and those administered 45 mg/kg showed the least increase. The serum testosterone concentrations of rats administered sildenafil citrate and the 45 mg/kg body weight of EESML were not significantly different ($P>0.05$).

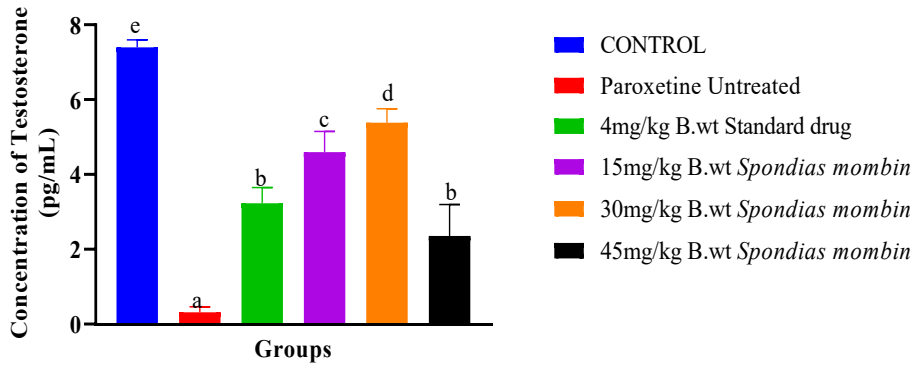


Figure 3: Testosterone Concentration in the serum of paroxetine- treated male Wistar rats after treatment with ethanol extract of *Spondias mombin* Leaf. The mean ± SEM of five measurements is shown by the data. Bars with various superscripts within the same group differ significantly from one another (P < 0.05).

Serum Troponin I Concentration in Paroxetine-Administered Male Wistar Rats Followed by Treatment with Ethanol Extract of *Spondias mombin* Leaf:

Figure 4 shows the Troponin I concentration in rats orally administered paroxetine for twenty-one days and various doses of EESML for the next seven days. Troponin I concentration was significantly (P < 0.05) lower in rats administered 15 mg/kg body weight of the ethanol extract of *Spondias mombin* leaf compared with the untreated group**.** Serum troponin I concentrations were similar (P>0.05) to those of the naive control group when *S. mombin* ethanol extract was administered at dosages of 30 and 45 mg/kg body weight. Furthermore, there was no significant difference (P>0.05) in Troponin I concentrations between rats treated with 15 mg/kg body weight of EESML and the group that received the standard drug.

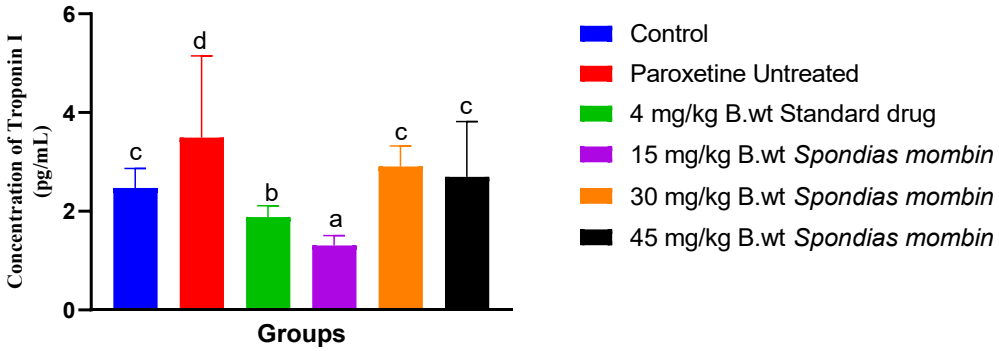


Figure 4: Serum Troponin I levels in paroxetine-induced male Wistar rats administered *Spondias mombin* leaf ethanol extract. Data represent the mean ± SEM of five measurements. Bars with various superscripts within the same group differ noticeably from each other (P < 0.05).

Serum lipid profile of paroxetine Administered Male Wistar Rats Followed by Treatment with the ethanol extract of *Spondias mombin* leaf:

Table 4 shows the serum lipid profile of male Wistar rats treated with *Spondias mombin* extract after receiving oral paroxetine. The negative control group had significantly higher serum cholesterol levels (P < 0.05) than the naive control group. Nevertheless, there was no significant difference (P>0.05) between the naïve control group and the 30 mg/kg b. wt. of the EESM group and the group that received sildenafil citrate.

The serum triglyceride level in the untreated (negative control) group was noticeably higher than that in the naive control group. Serum triglyceride concentrations were significantly reduced (P < 0.05) when the conventional medication or various extract doses were administered.

In contrast to the naive control group, the proportion of high-density lipoprotein cholesterol (HDL-C) in the serum of male Wistar rats administered with paroxetine decreased significantly (P<0.05). Rats treated with *Spondias mombin* extract showed significantly higher (P<0.05) serum HDL-C contents, despite no evident differences were observed between the treatment, reference medication, and naive control groups.

A significant (P<0.05) increase in the serum very low-density lipoprotein (VLDL) level was seen in paroxetine- treated but untreated (negative control) group in comparison to the positive control group. Administration of the various doses of *Spondias mombin* extract to the paroxetine- treated rats significantly reduced (P<0.05) serum VLDL concentration in group treated with extract, though the result depicted insignificant difference relative to naive control groups and group treated with standard drug.

The concentration of LDL significantly increased in the serum of the untreated group induced with paroxetine (negative control) relative to the naive control group. Conversely, Serum LDL levels were dramatically reduced (P<0.05) in the groups treated with *Spondias mombin* extract, although this reduction did not differ significantly (P>0.05) from the naive control group or the group receiving the standard medication.

The atherogenic indices of the result were depicted in Table 4. According to this table, the cardiovascular risk indices of rats in the untreated group were significantly higher (P<0.05) than

those in the other groups. There was little variation (P>0.05) in the rats' atherogenic index. administered standard drug and those administered extract, although both groups differed noticeably from the naive control.

Table 4: Lipid profile of paroxetine treated male Wistar rats following treatment with *Spondias mombin* extract

Groups	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-Cholesterol (mmol/L)	VLDL (mmol/L)	LDL- cholesterol (mmol/L)	Artherogenic index (mmol/L)
Control	22.74 ± 1.81^{b}	9.38 ± 0.12^{a}	8.09 ± 0.09^{b}	1.88 ± 0.02^{a}	12.77 ± 1.8^{a}	0.06 ± 0.01^{a}
Paroxetine Untreated	28.33 ± 1.07^{c}	13.82 ± 2.02^{b}	6.43 ± 0.26^{a}	2.23 ± 0.23^{b}	17.21 ± 0.40^{c}	0.32 ± 0.05^{b}
Paroxetine was treated with a standard drug	24.91 ± 1.35^{b}	10.12 ± 0.11^{a}	8.06 ± 0.09^{b}	1.79 ± 0.10^{a}	14.16 ± 1.19^{a}	0.09 ± 0.05^{a}
15mg/kg B. wt *S. mombin*	24.64 ±0.29^{b}	10.37 ± 0.83^{a}	8.12 ± 0.55^{b}	1.74 ± 0.17^{a}	15.78 ± 0.21^{b}	0.11 ± 0.02^{a}
30mg/kg B.wt *S. mombin*	21.34 ± 0.23^{a}	9.41 ± 0.32^{a}	7.78 ± 0.04^{b}	1.88 ± 0.07^{a}	11.68 ± 0.21^{a}	0.08 ± 0.02^{a}
45mg/kg B.wt *S. mombin*	23.10 ± 0.87^{b}	9.41 ± 0.29^{a}	7.96 ± 0.12^{b}	1.88 ± 0.05^{a}	13.26 ± 0.97^{a}	0.06 ± 0.02^{a}

Data represent the mean ± SEM of five measurements. Bars with distinct superscripts within the same group differ significantly from one another (P < 0.05)

4. DISCUSSION

For a male to achieve and sustain an erection, the penile blood vessels must dilate, the corpus carvenosum and spongiosum's veno-occlusive mechanism must be activated, and the trabecular smooth muscle must also relax (Ojo *et al*., 2019). This sequence is influenced by a decline in nitric oxide availability, which may result from reduced endothelial nitric oxide synthase (NOS) expression or performance and/or enhanced degradation of nitric oxide caused by different factors including psychogenic influences such as antidepressants. Selective serotonin reuptake inhibitors (SSRIs) such as paroxetine are frequently prescribed and generally better tolerated than older antidepressants. However, side effects such as sexual dysfunction limit patient acceptance of these medications (Cai *et al*., 2020). Paroxetine inhibits cytochrome P_{450} 2D6 and leads to low nitric oxide levels by inhibiting nitric oxide synthase (Horackova *et al*., 2025). Consequently, paroxetine administration alters markers of erectile and endothelial function (Muritala & Bewaji, 2021).

Phosphodiesterase 5 (PDE-5) has been suggested as a marker for erectile dysfunction (Zhu & Imperato-McGinley, 2017; Muritala, 2018). The gradual rise of the activity of PDE-5 following administration of paroxetine aligns with the observations of Muritala and Bewaji (2021). They reported that a 21-day paroxetine induction progressively increased PDE-5 activity in rats. This was also consistent with the finding of Zhu and Imperato-McGinley (2017), which revealed that PDE- 5 is a marker for erectile dysfunction. The progressive decline in PDE-5 activity in the penile organ following the administration of an ethanolic extract of S. mombin corroborates the results of Burnett (2008), who investigated pharmacotherapeutic approaches targeting PDE-5 to maintain penile health. The advent of PDE-5 inhibitors such as sildenafil underscored the importance of nitric oxide-mediated relaxation pathways in treating erectile dysfunction. PDE-5 inhibitors prevent cGMP degradation by inhibiting PDE-5 and maintaining elevated cGMP levels. Nonetheless, PDE-5 inhibitors cause other adverse effects, which include blocked nose, headache, visual disturbances, and indigestion (Ojo *et al*., 2019).

Nitric oxide plays an important role in mediating the penile vasculature changes essential for erection and is also hypothesized to promote penile smooth muscle relaxation via cyclic guanosine monophosphate, thereby contributing to physiologic erection (Liao *et al*., 2019). The progressive increase in nitric oxide concentration observed in rats treated with the ethanol extract of *S. mombin* is consistent with the findings of Karakus and Burnett (2020), who stated that PDE-5 inhibitors are not effective without inducing penile erection via nitric oxide release. Meanwhile, paroxetine-untreated rats' penile and cardiac tissues showed a decrease in nitric oxide levels, indicating decreased nitric oxide synthase activity, consistent with paroxetine's known inhibitory effect on this enzyme (Muritala & Bewaji, 2021).

Previous studies have demonstrated that modulation of arginase activity is linked to nitric oxide formation (Shin *et al*., 2016; Muritala, 2018). In cells, NOS and arginase enzyme compete for the common substrate L-arginine. Arginase facilitates the urea cycle's transformation of L-arginine into ornithine and urea (Mammedova *et al*., 2021). Studies have recognized the role of arginase in the treatment of sexual diseases (Adefegha *et al*., 2018; Ojo *et al*., 2019), resulting in decreased nitric oxide levels and elevated penile arginase activity. Accelerated L-arginine utilization may be responsible for increased arginase activity in tissues. Increased arginase activity and changes in endothelial nitric oxide synthase (eNOS) expression have been associated with reduced nitric oxide levels in erectile dysfunction. The findings of Adefegha *et al*. (2018) are consistent with the elevated arginase activity observed in the untreated group. In our study, the EESML suppression of arginase in both penile and cardiac tissues (Figures 2A and 2B) may represent an alternative way to enhance eNOS activity, thereby increasing nitric oxide bioavailability.

The elevated specific activities of serum 17β-hydroxysteroid dehydrogenase (17β-HSD) and 3β-

hydroxysteroid dehydrogenase after treatment with S. mombin ethanol extract are consistent with those of Wang *et al.* (2020), who found that erectile dysfunction results from inhibition of 17β- and 3β-HSD activity.

Low testosterone concentration is linked to erectile dysfunction in men. Testosterone is the main male hormone and indicator of male androgenicity (Ejike *et al*., 2019; Anderson *et al*., 2022). Rats treated with *Spondias mombin* ethanol extract showed increased testosterone levels, supporting the claim that the plant has aphrodisiac potential and may contain androgenic phytochemicals that boost the pituitary gland's ability to synthesize testosterone.

Elevated blood testosterone levels may be linked to the increased activity of serum 17β-hydroxysteroid dehydrogenase (17β-HSD) and 3β-hydroxysteroid dehydrogenase following treatment with ethanol extract of *Spondias mombin* and conventional medicine. Serum testosterone concentration is caused by residual 17β-HSD3 activity, 17β-HSD5 activity in extragonadal tissue, and Leydig cells converting high androstenedione levels into testosterone (Gomes *et al*., 2023). The results for the untreated groups were consistent with 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) enzyme deficiency, which is characterized by testosterone concentrations that are significantly low to normal and impaired testosterone synthesis (Mendonca *et al*., 2017).

Recent studies have demonstrated the connection between cardiovascular disorders and erectile dysfunction (Mostafaei *et al*., 2021; Cortese *et al.,* 2023). Cardiovascular disorders and erectile dysfunction are linked to numerous causes, such as elevated cholesterol and decreased HDL (Imprialos *et al.,* 2018). Men with erectile dysfunction and low testosterone levels have been reported (Ho *et al*., 2016). Elevation of total serum cholesterol and LDL are associated with decreased testosterone levels (Kloner *et al*., 2016). This is demonstrated by the study's finding that untreated paroxetine significantly increased low-density lipoprotein cholesterol levels. The experimental animals' levels of HDL-C, triglycerides, cholesterol, LDL-C, and VLDL were all markedly elevated by the *S. mombin* ethanol extracts. This suggests that *S. mombin* extract may suggest having a lipid-lowering effect and lower the risk of cardiovascular disease.

Increased Troponin I concentrations in the paroxetine-treated and untreated groups are consistent with the findings of Clerico *et al*. (2023). Their results showed that participants with erectile dysfunction had much higher levels of hs-troponin I (Hs-cTnI) than subjects without erectile dysfunction. He found that circulating quantities of hs-cTnI are linked to the presence and severity of erectile dysfunction, suggesting subclinical myocardial damage, even when the causal agents of cardiovascular disease, endothelial dysfunction, and heart failure are present. The effect of EESML was to reduce the concentration of Troponin I. This suggests that *the S. mombin ethanol extract may be able* to ameliorate cardiac dysfunction in rats. The progressive decrease in the atherogenic index of rats with increasing dosage is consistent with the lipid profile results and corroborates the claim that the plant could also play a lipid-lowering role and reduce the risk of cardiovascular disease.

CONCLUSION

Ethanol extract of *Spondias mombin* leaves significantly improved erectile function markers, indicating its potential for natural treatment of erectile dysfunction. The effects of *S. mombin* ethanol extract on lipid profile, atherogenic index, and serum Troponin I concentration also indicated that the extract may reduce the risk of cardiovascular disease.

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Ethical Statement:

Approval to conduct this study was provided by the Ethical Committee of the University of Ilorin, Ilorin, Nigeria (No.: 1079, date: 16th August 2024). In addition, this study was conducted in accordance with the local legislation and institutional requirements of a research protocol, as per the Ministry of Health, Nigeria (Form number 04/2025).

Author Contributions:

All authors have reviewed the final version to be published and have agreed to be accountable for all aspects of the work in the SJUOZ. Concept and design: H.F.M; Data Acquisition: H.F.M., Z.B.L; Analysis: Z.B.L., H.N.M., E.A.D; Interpretation and visualization of data: Z.B.L., H.F.M., L.A.Q.; Writing first draft of the manuscript: Z.B.L, H.F.M.; Editing and Paraphrasing of the manuscript: H.N.M, and Z.B.L.

Conflict of Interest:

The authors have no conflicts of interest.

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