J. Phytomol. Pharmacol., 3(1), 32-43, 2024
https://doi.org/10.56717/jpp.2024.v03i01.025
ISSN(Print): 2957-5818, ISSN(Online): 2958-6224

Journal of Phytomolecules & Pharmacology

Research Article

Diabetes impairs male sexual behaviour: Ameliorative role of ethanol leaf extract of *Alchornea cordifolia* and its antihyperlipidemic activity

Sunday Augustine Ejeh[1*], Hyacinth Adakole Abu[2], Jerry Ngutor Abenga[3], Samson Eneojo Abalaka[4], Ndidi Glory Enefe[1] and Ishaku Eugenia[5]

1. Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria, Nigeria.
2. Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria.
3. Department of Veterinary Pathology, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria.
4. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria, Nigeria.
5. Department of Public Health, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria, Nigeria.

Abstract

The use of alternative medicinal approaches in the management of diabetes-associated reproductive dysfunction has become imperative, hence, this study was designed to evaluate the ameliorative potential of *Alchornea cordifolia* in diabetic-induced sexual behavioural dysfunction and its antidyslipidemic activity. Twenty-five male diabetic rats were divided into five groups of five rats each. Group I received distilled water, Groups II and III received 100 and 200 mg/kg of the extract, respectively, Group IV received 5 mg/kg metformin sulfate and Group V was diabetic but received no treatments. Treatment was done daily by oral gavage for 28 days. Hypoglycemic activity, lipid profiles, and sexual behaviors such as mount and intromission frequencies, mount, intromission and ejaculation latencies, and post-ejaculation interval were evaluated according to standard procedures. The results showed significantly reduced plasma glucose concentrations in extract exposed groups and the standard antidiabetic group (127.6 ± 3.33^{b}, 118 ± 10.15^{b}, and 114.6 ± 11.74^{b}) compared to the diabetic untreated group (270.6 ± 82.64). Again, there was a significant reduction in total serum cholesterol concentration ($99.93 \pm 5.19^{**}$) in the 200 mg/kg extract compared to the diabetic untreated group (130.8 ± 6.76), lipid profiles with elevated mount and intromission frequencies and ejaculation latency. Reductions in mount and intromission latencies were also recorded in extract-exposed groups. The findings showed that the extract possessed antidiabetic and antihyperlipidemic activities and could enhance sexual performance and increase sexual satisfaction in animals. Therefore, the treatment of diabetic male Wistar rats with the extract, ameliorated reproductive damage induced by diabetes and improved sexual behaviors in those animals.

Article Information
Received: 11 July 2024
Revised: 12 August 2024
Accepted: 14 August 2024
Published: 19 September 2024

Academic Editor
Prof. Dr. Marcello Iriti

Corresponding Author
Prof. Dr. Sunday Augustine Ejeh
E-mail: sunday.ejeh@uniabuja.edu.ng
Tel: +2348079402362

Keywords
Alchornea cordifolia, diabetes, mating, sexual behavior, streptozotocin.

1. Introduction

There is a growing concern over the spate of male infertility [1], especially when associated with dysfunctions in male sexual behavior [2]. Disorders of male sexual behavior could take the form of a lack of sexual

stimulation due to poor autonomic and somatosensory signals, a disorder of ejaculation, erectile dysfunction, and a disturbance of libido and orgasm [3, 4]. Kumar and Singh, [5] reported that about 48.5 million couples were infertile as of 2010, with 45-50% resulting from male factor infertility probably due to dysfunctional sexual behavior. However, the current estimate of men with erectile dysfunction is 186 million, which may increase to about 322 million by 2025 worldwide [6]. Erectile dysfunction is the failure to achieve and maintain an erection, strong enough to penetrate and produce sexual satisfaction during sexual intercourse [7]. This has been associated with increasing age and chronic diabetes as major risk factors [8]. Moreover, half the population of diabetic men have erectile dysfunction [9], with 35-75% of them experiencing erectile dysfunction worldwide [10]. The mechanism by which diabetes results in sexual dysfunction, particularly erectile dysfunction, is related to the dysregulation of several physiological factors, including psychogenic, hemodynamic, neurogenic, and hormonal changes, as well as the atrophy of the corpus cavernous muscle [11]. Others may include micro and macrovascular complications such as cardiovascular, neuropathy, and hypogonadism secondary to diabetic cases in men [12]. Again, dyslipidemia such as increased circulating cholesterol and triglyceride with low-density lipoproteins has been implicated in several male sexual dysfunctions [13]. Furthermore, increased endothelial dysfunction may impair penile arterial blood flows necessary for the maintenance of erection whereas neuropathy may be responsible for the disruption of somatosensory signals from the penis to the reflexogenic center in the brain thereby preventing smooth muscle relaxation to enhance and sustain an erection [14]. According to Ismail et al [15], increased endothelial dysfunction in diabetics may be due to elevated reactive oxygen species, which decreases the bioavailability of nitric oxide resulting in defective relaxation of the smooth muscle of corpora cavernous. This will further lead to an imbalance in vasodilative and vasoconstrictive extracellular pathways favoring vasoconstriction and poor erection [9].

With the increasing cases of diabetic complications, including reproductive dysfunctions among men, and the multifactorial causes of erectile and other sexual dysfunctions, there is a need for a broader approach to treating and managing diabetes-associated male sexual dysfunctions. Such approaches may include changes in lifestyle in terms of increased physical exercise, weight loss, and healthy dietary intake [16], as well as glycemic control, and pharmacotherapy via phosphodiesterase 5-inhibitor administration [17]. A phosphodiesterase 5-inhibitor pharmacotherapeutic drug like Sildenafil is known to enhance erection by inhibiting the enzyme's phosphodiesterase action with a prolongation of the activity of cyclic guanosine monophosphate in the erectile muscles hence, the sustenance of erection [18]. Despite the success story recorded by this agent in improving erection in patients with erectile dysfunctions, evidence of low responses to this drug also exists [19]. However, phosphodiesterase 5-inhibitor reportedly causes severe myocardial infarction, chest pain, and cardiac arrhythmias in patients treated with this agent [20]. Therefore, the drug has also become motivating factor for researchers in the search for alternative traditional plant products with minimal side effects but improved efficacy and potency [21].

Alchornea cordifolia (*A. cordifolia*) is a popular medicinal plant shrub found within the tropical forests of East, West, and Central Africa, usually in wet or swampy areas as well as in acidic soils [22]. The plant reportedly has some bioactive agents such as flavonoids, terpenoids, alkaloids, tannins, saponins, alchorneine, alchornidine, glycosides, and several other compounds [23]. There are reports of the use of the leaves, stems, bark, and roots of the plant to treat various ailments including rheumatic pain, respiratory disorders, and reproductive diseases in Nigeria [24]. Again, Ngaha-Njila et al. [25] highlighted the role of the plant in the enhancement of sexual behavior in sexually inexperienced animals. However, scientific investigations into the ameliorative effects of the plant on sexual dysfunctions induced by diabetes mellitus and its antihyperlipidemic activity are scanty to warrant the present work. Therefore, this study aimed to investigate the possible ameliorative role of ethanol leaf extract of *A. cordifolia* on sexual behavior in streptozotocin-induced reproductive dysfunctions and its antidyslipidemic activity in male Wistar rats.

https://doi.org/10.56717/jpp.2024.v03i01.025

2. Materials and methods

2.1. Collection and processing of plant materials

Leaves of *A. cordifolia* were harvested during the dried season between November and December 2023 from the Aikplia-Ugbokolo district, Okpokwu Local Government area of Benue State, Nigeria. Identification and authentication of the plant were carried out at the Department of Botany, Faculty of Biological Sciences, Joseph Sarwuan Tarka University, Makurdi, Benue State by Dr. Okoh Thomas where voucher number FUAM/BOT/ HERB/02781 was deposited for future reference. The harvested leaves were then washed thoroughly to remove impurities and other contaminants under fast-flowing tap water before air-drying at room temperature and pressure before pulverization into a fine powder ready for extraction. Soxhlet apparatus was used for the extraction of the powdered leaf using absolute ethanol as the extraction solvent as previously described [26].

2.2. Inductions of diabetes using streptozotocin in male rats

Diabetes was induced in the male Wistar rats after overnight fasting by injecting a single dose of 45 mg/kg of fresh streptozotocin (Sigma-Aldrich Corp. St. Louis, Mo. USA) in cold 0.1M citrate buffer at pH 4.5 intraperitoneally. After 48 hours, blood samples from the tail vein were collected to measure the blood glucose concentrations using an Accu-chek glucometer (Accu-chek GB, Roche Mannheim Germany). Animals with blood glucose levels above 200 mg/dl were selected for this study.

2.3 Experimental animals

Twenty-five (25) sexually matured male Wistar rats weighing between 180 - 200 g and twenty-five (25) sexually receptive female Wistar rats were sourced from the animal house of the Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria. The rats were paired in a plastic cage measuring 60 X 20 X 30 cm with proper ventilation. The rats were allowed to acclimatise for two weeks under standard temperature and relative humidity with a 12-hour day/light cycle. During this period and the entire treatment cycle, the rats had access to fresh drinking water and were fed on standard animal feeds (Super Deluxe Animal, Premier Feed Mills Co. Ltd., Kaduna, Nigeria) *ad libitum*. Ethical clearance was obtained from the Ethics Committee on Animal Use and Care of the University of Abuja (ECAUCUA) with approval number ECAUCUA /2022/005.

2.4. Experimental grouping

Twenty-five male diabetic rats were randomly divided into five groups of five animals each as follows:

Group I: Control (non-diabetic)
Group II: Diabetic control
Group III: Diabetic + 100 mg/kg (extract)
Group IV: Diabetic + 200 mg/kg (extract)
Group V: Diabetic + 5 mg/kg (metformin sulfate)

Administration of the extract and standard antidiabetic drug was done daily for 28 days with water and feed provided *ad libitum.* Weekly plasma glucose levels were measured using Accu Check glucometer® following the principle of oxidase reaction [27].

2.5 Estimation of the lipid profile of streptozotocin-induced diabetic male Wistar rats exposed to ethanol leaf extract of Alcorenea cordifolia.

Twenty-four hours after the last dose administration of the extract and standard drug, 4 mL of blood samples were collected from the lateral orbital plexus of the eye into a plain test tube containing a serum coagulator. The tubes were slanted for a few hours to allow coagulation to take place, thereafter, the supernatant was harvested and stored in the refrigerator for the analysis of the lipid profile. The level of serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDLc), and high-density lipoprotein cholesterol (HLDc) were evaluated by spectrophotometric methods using commercial kits (Spectrum Diagnostic, MDSS GmbH, Schiffgraben 41, 30175, Hannover Germany) and according to the manufacturer's instructions.

2.6. Sexual behavior study

Female rats were screened using cytological technique and those detected to be in estrus were selected and used in this study. After 28 days of treatment, the effect of the extract of *A. cordifolia* and standard antidiabetic drug on the male sexual behaviour of the rats was determined after being paired with the receptive female, according to the method described by [28] with some modifications. For this sexual behavioral study, an open field arena of aluminium rat cages of 60 X 20 X 30 cm was used, the same

https://doi.org/10.56717/jpp.2024.v03i01.025

Table 1. Measurement of weekly plasma blood glucose concentrations of streptozotocin-induced diabetic male albino Wistar rats exposed to ethanol leaf extract of *Alcornea cordifolia*

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
Control	109.6±3.47	113.2±1.66	99.0±1.38	102.2±2.15	104.6±2.62[b]
Diabetic control	79.60±7.15	360.8±37.19	269.2±60.07	299.2±69.53	270.6±82.64
Extract (100 (mg/kg)	86.2±7.74	218.0±43.07	182.0±28.76	166.4±24.89[b]	127.6±3.33[b]
Extract (200 mg/kg)	81.2±6.95	258.4±36.38	182.6±52.58	178.4.2±15.16	118±10.15[b]
Metformin (5 mg/kg)	80.6±4.53	277.4±26.99	221.4±34.69	138.2±10.86[b]	114.6±11.74[b]

Data are presented as Mean ±SEM (N=5). Values with superscripts are statistically significant ($p<0.05$) compared to the diabetic untreated group following post hoc Dunnett's multiple comparisons.

as for housing the animals during the experiment. The cages were emptied of bedding and moved to the sexual behavioral room. The video camcorder, Nikon Coolpix L341 (Nikon Imaging, USA) used for video capturing of sexual behavior was positioned 90 cm above the centre of the open cage floor. The entire arena of the cage in the zone of the camera's view was captured from this position. The video camcorder was connected to a Samsung laptop, (Samsung Electronics, South Korea). The behaviour observation tests started at 3.00 hours after the onset of darkness and were performed under red illumination and by pressing the space bar key on the computer keyboard; the test starts and ends manually. The animal's sexual behavior inside the cage arena was manually scored from the recorded videos by two investigators blinded to experimental groups with good concordance. Before the start of the actual sexual behavioral test, individual male Wistar rats were placed in the observation cage and allowed for 10 min to acclimate the cage environment. Each sexual behavior test was initiated with the introduction of a receptive female Wistar rat dropped silently from one side of the cage as the stimulus, and then an observable video was recorded for 20 minutes. Each male had one female and no repetition was done in this study.

1. In this sexual behavioral experiment, the parameters studied were as described by Mos et al. [28] and include the following:
2. Mount latency (ML): The time from the introduction of female Wistar rats to the occurrence of the first mount (in seconds).
3. Mount frequency (MF): The total number of mounts within 30 minutes of the test.
4. Intromission latency (IL): The time from the introduction of a female Wister rat to the occurrence of the first intromission (in seconds).
5. Intromission frequency (IF): The number of intromissions within 30 minutes of the test
6. Ejaculation Latency (EL): The time interval between the first intromission and ejaculation.
7. Post ejaculation Interval (PEI): The time from the first ejaculation to the next mount or intromission

To further assess the effects of the extract on the efficiency of copulatory behavior, the intromission ratio was calculated using the expression:

$$\text{Intromission ratio} = \frac{\text{Intromission}}{\text{Number of intromission} + \text{Number of mount}}$$

The sexual behaviour testing was done on all Wistar rats in each group.

2.7. Statistical analysis

Data obtained from this study are presented as mean ± SEM and the difference between treatment groups were analysed using One-way analysis of variance (ANOVA) followed by post hoc Dunnett's comparison test for statistical significance at $p<0.05$ with the aid of GraphPad Prism version 7.0.

3. Results

3.1 Measurement of weekly blood glucose concentrations of *streptozotocin-induced diabetic male Wistar rats exposed to ethanol leaf extract of Alcornea cordifolia*

The results of the treatment on diabetic male Wistar rats of the extract in weekly blood glucose levels were presented in Table 1. It was observed that the increased blood glucose level induced by the damaging effects of streptozotocin on the beta cells of the pancreas was significantly ($p<0.05$) lowered by the standard antidiabetic agent and 100 mg/kg extract-

https://doi.org/10.56717/jpp.2024.v03i01.025

Table 2. Serum lipid profile of ethanol extract of *Alchornea cordifolia* leaves exposed to streptozotocin-induced diabetic male Wistar rats.

Parameters	Diabetic Untreated	Control	Extract (100 mg/kg)	Extract (200 mg/kg)	Metformin (5 mg/kg)
Tot. cholesterol (mg/dl)	130.8±6.76	125.2±6.50	114.3±5.80	99.93±5.19**	136.8±5.56
HDLc (mg/dl)	31.04±1.36	35.49±1.12	33.18±0.82	23.25±2.41	45.26±4.56**
LDLc (mg/dl)	71.47±8.95	69.58±6.52	55.35±5.37	52.58±1.39	61.75±7.24
Triglyceride (mg/dl)	141.2±14.11	100.4±4.57	129.0±15.36	120.5±15.80	148.9±7.14

Values with asterisk are statistically significant ($p<0.05$) compared to the diabetic untreated group following post hoc Dunnett's multiple comparison test. Values are presented as Mean ± SEM. N=5. Tot. Cholesterol= total cholesterol; HDLc= High Density Lipoprotein cholesterol; LDLc= Low Density Lipoprotein Cholesterol

treated groups at week three of the experimental period (166.4 ± 24.89^{b}; $178.4.2 \pm 15.16$) when compared with the diabetic untreated group (269.2 ± 60.07). Furthermore, at week four of the treatment, blood glucose concentrations were significantly ($p< 0.05$) reduced in the extract-treated and standard antidiabetic groups (100 and 200 mg/kg and 5 mg/kg) respectively, compared with the diabetic untreated group.

3.2 Effects of ethanol extract of Alchornea cordifolia leaf in streptozotocin-induced diabetic male rats on serum lipid profile.

Table 2 showed the serum lipid profile of extract-exposed diabetic male Wistar rats. It was observed that streptozotocin induced increased total cholesterol, low-density lipoprotein cholesterol and triglyceride with a decreased high-density lipoprotein cholesterol. However, exposure of the diabetic male Wistar rats to *A. cordifolia* extract significantly ($p<0.05$) decreased total cholesterol at 200 mg/kg compared to the diabetic control. Furthermore, there was a decrease in low-density lipoprotein, and triglyceride concentrations in the extract-exposed groups compared to the diabetic control. However, the results were not statistically significant ($p>0.05$). It was further observed that the value of the high-density lipoprotein cholesterol was elevated in the extract-exposed group (100 mg/kg), though, this was not statistically significant ($p> 0.05$).

3.3 Effects of ethanol extract of Alchornea cordifolia leaves exposed to streptozotocin-induced diabetic male Wistar rats on Mount frequency

In the control as well as the diabetic groups exposed to 100 and 200 mg/kg body weight of *A. cordifolia*, the mount frequency behaviour was in increased succession and was followed by pelvic thrusting (intromission) until ejaculation. This behaviour was not so common in the diabetic and untreated groups. Our result revealed a significant ($p<0.05$) increase in the number of mounts in the 100 mg/kg body weight *A.cordifolia* treated group compared to the diabetic control group and between the standard drug (metformin) and the treated group. Also, there was a significant increase ($p<0.05$) in the 200 mg/kg body weight of *A. cordifolia* diabetic-treated group compared to the diabetic control group. Although there was an increase in mount frequency, comparing the normal control group with the 100mg/kg and 200mg/kg groups, these increases were not statistically significant ($p>0.05$). No significant ($p>0.05$) difference was also seen between the diabetic and standard drug (metformin) treated group and with the 200 mg/kg body weight of the *A. cordifolia* treated group (Fig. 1).

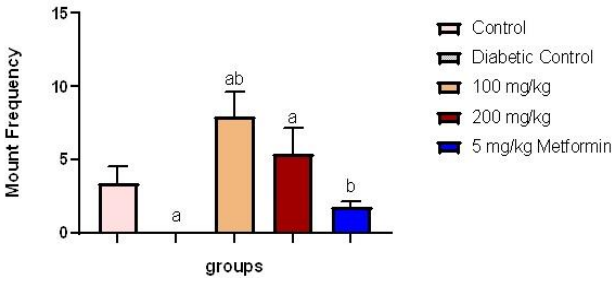

Figure 1. Effects of ethanol leaf extract of *Alchornea cordifolia* on streptozotocin-induced diabetic male Wistar rats on mount frequency (Bars with the same superscripts are statistically significant at $p<0.05$ after Post hoc Dunnett's multiple comparison test).

3.4. Effects of ethanol leaf extract of Alchornea cordifolia on streptozotocin-induced diabetic male Wistar rats on mount latency.

https://doi.org/10.56717/jpp.2024.v03i01.025

Concerning the mounting latency, the ethanolic leaf extract of *A. cordifolia* significantly ($p<0.05$) affected this sexual behaviour in streptozotocin-induced diabetic male Wistar rats (Fig. 2).

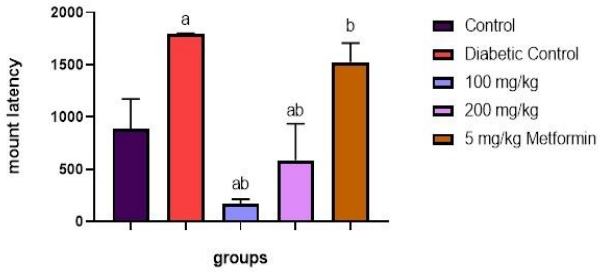

Figure 2. Effects of ethanol leaf extract of *Alchornea cordifolia* on streptozotocin-induced diabetic male Wistar rats on mount latency. (Bars with the same superscripts are statistically significant at $p<0.05$ after post hoc Dunnett's multiple comparison tests).

Specifically, the mounting latency recorded from the diabetic male Wister rats with no treatment and the diabetic rats with 5 mg/kg body weight metformin-treated groups was significantly ($p<0.05$) increased compared to the 100 mg/kg ($P<0.001$) and 200 mg/kg, respectively, in diabetic male Wistar rats treated with ethanolic leaf extract of *A. cordifolia*. Comparing the effects of the two doses of ethanolic leaf extract of *A. cordifolia* and metformin-treated groups on mount latency sexual behaviour in diabetic male Wistar rats, our results showed significantly an ameliorative effect in favour of *A. cordifolia* at 100 mg/kg and 200 mg/kg reduced the mount latency compared to 5 mg/kg body metformin did.

3.5. Effects of ethanol leaf extract of Alchornea cordifolia in streptozotocin-induced diabetic male rats on the frequency of intromission

On intromission frequency, male Wister rats in the control as well as in the 100mg/kg and 200 mg/kg body weight of *A. cordifolia* treated diabetic Wistar rat groups had increased intromission compared to the diabetic without treatment, and the diabetic with 5 mg/kg metformin-treated groups (Fig. 3). Post-hoc multiple comparisons showed a significantly ($P<0.05$) increased intromission comparing the 200 mg/kg body weight *A. cordifolia* treated diabetic rat group to the diabetic control group ($P<0.05$) and the diabetic group treated with 5 mg/kg body weight of *A. cordifolia* (Fig. 3).

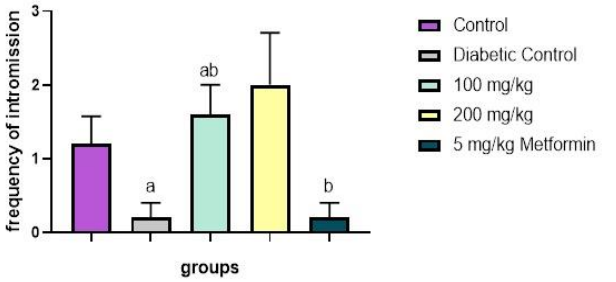

Figure 3. Effects of ethanol leaf extract of *Alchornea cordifolia* in streptozotocin-induced diabetic male Wistar rats on the frequency of intromission. (Bars with the same superscripts are statistically significant at $p<0.05$ after Post hoc Dunnett's multiple comparison test).

3.6. Effects of ethanol leaf extract of Alchornea cordifolia on streptozotocin-induced diabetic male Wistar rats on intromission latency

Our results also showed that diabetes-induced increased intromission latency was observed in the diabetic untreated group. Interestingly, exposure of diabetic male Wistar rats to *A. cordifolia* at 100 mg/kg and 200 mg/kg significantly reduced the atency induced by diabetes (Fig. 4).

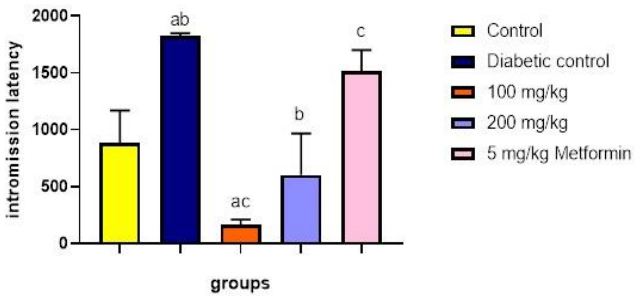

Figure 4. Effects of ethanol leaf extract of *Alchornea cordifolia* in Streptozotocin-induced diabetic male Wistar rats on intromission latency. (Bars with the same superscripts are statistically significant at $p<0.05$ after post hoc Dunnett's multiple comparison test).

Interestingly, rats exposed to 100 mg/kg of the extract showed a significant ($p< 0.05$) reduction in intromission latency compared with the standard antidiabetic agent (Metformin) used in this study. Also, comparing the effects of the extract exposed rats and metformin-treated groups on intromission latency to the diabetic untreated male rats, our results showed an ameliorative effect in favor of *A. cordifolia* as 100 mg/kg significantly ($P<0.05$) reduced the intromission latency compared to 5 mg/kg metformin group (Fig. 4).

https://doi.org/10.56717/jpp.2024.v03i01.025

3.7. Effects of ethanol leaf extract of Alchornea cordifolia in streptozotocin-induced diabetic male Wistar rats on ejaculation latency

Concerning the effects of the extract of *A. cordifolia* on the ejaculation latency of the diabetic male Wistar rats, no significant (P>0.05) changes were seen across the groups. However, treatment of these diabetic Wistar rats with extract of *A. cordifolia* at both doses used for this experiment (100 mg/kg and 200 mg/kg) reduced the ejaculation latency (Fig. 5).

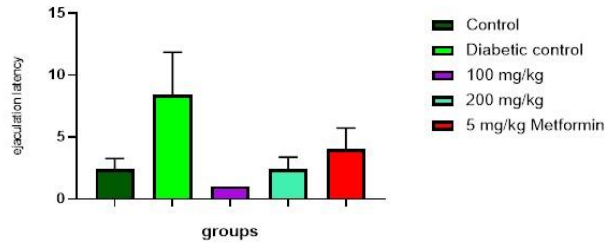

Figure 5. Effects of ethanol leaf extract of *Alchornea cordifolia* in streptozotocin-induced diabetic male rats on ejaculation latency.

3.8. Effects of ethanol leaf extract of Alchornea cordifolia in streptozotocin-induced diabetic male Wistar rats on post-ejaculation interval

Figure 6 showed the results of the effects of the extract on the post-ejaculation interval in diabetic male Wistar rats. It was observed that there were no significant (P>0.05) changes across the treated groups. The observations notwithstanding, there were decreases in 100 and 200 mg/kg of the extract-treated groups compared with the other groups.

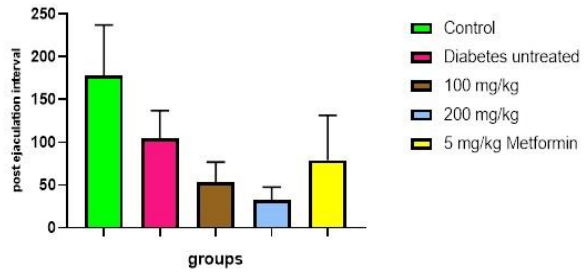

Figure 6. Effects of ethanol leaf extract of *Alchornea cordifolia* in streptozotocin-induced diabetic male Wistar rats on post-ejaculation interval.

3.9. Evaluation of intromission ratio of the streptozotocin-induced diabetic male Wistar rats exposed to ethanol leaf extract of Alchornea cordifolia.

To evaluate the impact of diabetes and the effects of the extract on copulatory behavioural efficiency, the authors calculated the intromission ratio using the standard formula. The results showed that diabetes reduced the intromission ratio, which was elevated by the administration of the extract. However, no statistical (P>0.05) changes were recorded when subjected to a post hoc multiple comparison test as presented in Fig. 7.

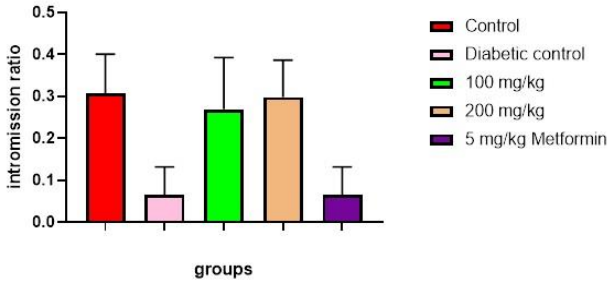

Figure 7. Effects of ethanol leaf extract of *Alchornea cordifolia* in streptozotocin-induced diabetic male Wistar rats on intromission ratio

4. Discussion

Diabetes mellitus has become a major global non-infectious disease of reproductive public health concern [29]. The disease is known for its multiorgan and multisystem complications, including neuropathy, nephropathy, cardiovascular disorders, retinopathy, and male reproductive dysfunctions [30]. The increased incidence of diabetes among young people of reproductive age has raised serious concerns about the impact of the disease on reproduction, including diabetes-related infertility [31]. In this study, exposure of diabetic male Wistar rats to ethanol extract of *A. cordifolia* significantly ($p< 0.05$) reduced the blood glucose levels, indicative of antidiabetic activity. This agreed with the report of [32] and the result of an *in vitro* antidiabetic study by [33], which recorded improved glycemic levels of the test groups. The antidiabetic property exhibited by the plant in this study was attributable to the presence of its phytochemicals, especially phenols and flavonoids [32, 33]. Furthermore, diabetic-related cardiovascular complications are currently a public health concern among individuals suffering from diabetes [34]. It has been demonstrated that some risk factors, such as hypertension, coronary artery disease and dyslipidemia are attributable to diabetes mellitus [35]. This study demonstrated that diabetes induced by

https://doi.org/10.56717/jpp.2024.v03i01.025

streptozotocin greatly increased serum total cholesterol concentration, low-density lipoprotein, and triglyceride and decreased high-density lipoprotein concentration. Conversely, an increased serum cholesterol concentration has been shown to enhance insulin resistance with a characteristic decrease in vascular nitric oxide levels resulting in a reduction in the vasodilation of penile arteries necessary for erection and hence, a decrease in fertility [36]. However, the exposure of these groups to the extract significantly ($p<0.05$) lowered the total cholesterol level, particularly at 200 mg/kg of the extract. Furthermore, elevated serum cholesterol in patients with diabetes has been implicated in devastating sexual behavioural dysfunctions including erectile and ejaculatory disorders [13]. It has been demonstrated that male infertility associated with diabetes occurs at different levels of the organ system to alter sexual behaviour, erection, and ejaculation with subsequent impairment of the hypothalamus-pituitary-gonadal activities and sexual desire [37, 38]. The use of plants and their products in the remediation of various medical conditions, including reproductive abnormalities among different ethnic groups and societies, has continued to arouse the interest of researchers in this field [39]. As such, the authors demonstrated the potential ameliorative effects of the extract in streptozotocin-induced diabetic Wistar rats on male sexual behaviours. Our results revealed a significant increase in mount and intromission frequencies of the extract-treated groups compared to the diabetic untreated group. Our report was in agreement with the work of Njile et al. [40], who showed that the administration of methanol leaf extract of *A. cordifolia* at different doses significantly improved sexual desire, vigour, performance, and libido of the treated male rats. However, sexual behaviour was not altered by any disease condition/agents in their study contrary to the present work in which male sexual behaviour was impaired by hyperglycemia.

The number of mounts and the rate of intromissions are important indices for male sexual performance, desire, strength and potency assessments [41]. It could be infered from the results of this study that the administration of this extract could be used to ameliorate erectile and ejaculatory dysfunctions associated with diabetic complications. This followed the fact that intromission frequency is an indication of the efficacy and efficiency of erection, penile orientation, and the enhancement of ejaculatory reflex [40]. Interestingly, reduced nitric oxide synthesis and release, and dysfunction of the endothelial membrane of penile vessels are responsible for poor maintenance of erection and ejaculation in diabetic patients [12]. Therefore, it could be hypothesized that the action of the plant in this study may be linked to the ability of the extract to increase nitric oxide synthesis and the improvement of the endothelial membrane of penile vessels. Furthermore, the increase in intromission frequency recorded in the *A. cordifolia* treated groups was an indication that the extract may possess bioactive ingredients that stimulate or alter erectile reflex at the peripheral or central nervous system, which coordinate the activity of erectile muscles (cavernous muscle), since, intromission cannot occur without proper initiation and sustenance of erection [40]. Also, the presence of important phytochemicals such as saponins and alkaloids, which are known to enhance vasodilation of penile blood vessels [42], with improved arterial blood flows to the penile muscle and resultant erections [41]. Furthermore, the reduction in lipid profile, particularly decreased cholesterol, triglycerides and low-density lipoprotein by the extract in this study might have been responsible for the recorded improved sexual activity. Increased serum cholesterol and triglycerides are known to promote atherosclerotic lesions within smaller arteries, particularly, penile arteries with consequential endothelial damage [13]. The above improvement in intromission and mount frequency further attests to a possibly increased endothelial nitrous oxide production and enhanced vasodilation of penile arteries. Therefore, the authors believe that the action of *A. cordifolia* extract might have been due to increased nitric oxide synthesis and the inhibition of phosphodiesterase enzymes. However, the assertion remains to be investigated. Similarly, the extract could be used to improve sexual motivation, performance, and vigour in diabetic patients.

Mount and intromission latencies were used as indicators of sexual arousal and motivation [42, 43]. The evaluation of sexual behavior in this study showed that the leaf extract of *A. cordifolia*

https://doi.org/10.56717/jpp.2024.v03i01.025

significantly lowered the mount and intromission latencies compared to the standard antidiabetic drug (100 mg/kg Metformin). The result was in agreement with the reports of Njile et al. [40] and Boublata et al. [44] that demonstrated the aphrodisiac activity of both methanol extract of *A. cordifolia* and ethanol leaf extract of *Cleome Arabica* on senescent and sexually naïve male rats. The improvement in sexual performance and motivation observed in this work may be linked to the presence of bioactive compounds such as flavonoids and steroids, which are known to enhance androgenic activities [45], particularly testosterone, and other neurotransmitters responsible for the initiation and sustenance of erection [46].

Diabetes-induced reproductive functional alterations and sexual behaviour deterioration are linked to an increased generation of free radicals, impaired nitric oxide (NO) synthesis, and a dysfunctional nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway [13] as well as the resultant upregulation of Transforming Growth Factor β1/Smad signalling [47]. Therefore, the improvement in sexual performance and motivation reported herein might be associated with the ability of the metabolites present in the extract to effectively scavenge excessive free radicals, increase nitric oxide and alter the NO-cGMP pathways by lowering blood hyperglycemia. Many medicinal plants including *A. cordifolia* have been known for their anti-oxidant effects [39, 40, 44].

Another male reproductive dysfunction linked to diabetes mellitus is ejaculatory dysfunction secondary to autonomic neuropathy syndrome [48], which results in anomalies ranging from premature ejaculation, failure of ejaculation, and retrograde ejaculation [49], among others. The authors reported herein a significantly ($p<0.05$) increased ejaculation latency following the treatment of diabetic male Wister rats with 200 mg/kg body weight of *A. cordifolia* extract compared with the diabetic control and standard antidiabetic group that received a known antidiabetic drug (Metformin). This indicated sexual pleasure and performance [50] as well as inducing ability of *A. cordifolia* extract, which further showed the aphrodisiac activity of the extract. The findings also corroborated the results of Njile et al [44] on elevation of ejaculation latency in senescent and sexually naive male Wistar rats following *A. cordifolia* administration at 200 mg/kg and 400 mg/kg doses, respectively. According to Malviya et al. [51] any substance with the ability to stimulate sexual reflexes such as ejaculation could be considered an aphrodisiac. To further demonstrate the aphrodisiac activity of the plant and to substantiate the earlier report of Njile et al [40], the authors monitored the post-ejaculatory interval and their findings revealed a reduction in this parameter in the extract-treated groups compared to the normal and diabetic control groups. Although the results were not statistically significant ($p>0.05$), it however, presented a clinical relevance, since the reduced post-ejaculatory interval is an indication of improved sexual libido, potency, and decreased exhaustion in the first series of mating [52]. Therefore, *A. cordifolia* possesses the potential to enhance sexual behaviour and performance, including diabetes mellitus-induced reproductive dysfunction to support the use of this aphrodisiac plant as an alternative to synthetic therapy [53].

5. Conclusions

The study demonstrated the antidiabetic and antihyperlipidemic activity of *A. cordifolia* and its propensity to improve sexual behavioural parameters in diabetic male Wistar rats. The results showed that the ethanol extract of *A. cordifolia* possesses the potential to restore glycemia, improve the lipid profile and sexual activity of male individuals suffering from reproductive problems associated with diabetes and enhance male fertility. Though, this study did not evaluate the mechanism of action of the extract in the improvement of the parameters measured, however, the findings call for more investigations to further elucidate the reproductive potentials of *A. cordifolia* in diabetic animals.

Institutional review board statement (Ethical statement)

Ethical clearance was sorted and received from the Ethics Committee on Animal Use and Care of the University of Abuja (ECAUCUA) with approval number ECAUCUA /2022/005.

Authors' contributions

Conceptualization, S.A.E., H.A.A., J.N.A.; Data

https://doi.org/10.56717/jpp.2024.v03i01.025

collection and analysis, S.A.E., H.A.A., J.N.A., N.G.E., S.E.A., I.E.; Prepared the manuscript, S.A.E.; Reviewed the manuscript, H.A.A., J.N.A., N.G.E., S.E.A.

Acknowledgements

Authors acknowledged the financial contribution of centre for Sponsor Projects through TETFund Institutional Research Grant, Dr Oyelewo A for critically reviewing the manuscript and Mr. Vincent Upev for his technical support.

Funding

This research work received financial support from the Tertiary Education Trust Fund (TETFUND) under her Institutional-Based Research Grant (TETF/DR&D/CE/UNI/ABUJA/IBR/2021/VOL.1) at the University of Abuja, Abuja Nigeria.

Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest

References

1. Eisenberg, M.L.; Chen, Z.; Ye, A.; Buck, R.; Louis, G.M. Relationship between physical occupational exposures and health on semen quality: data from the longitudinal investigation of fertility and the environment (LIFE) study. Fertil Steril. 2015, 103, 1271–1277. https://doi.org/10.1016/j.fertnstert.2015.02.010.
2. Lotti, F.; Corona, G.; Castellini, G.; Maseroli, E.; Fino, M.G.; Cozzolino, M.; Maggi, M. Semen quality impairment is associated with sexual dysfunction according to its severity. Human Reproduction (Oxford, England). 2016, 31(12), 2668–2680. https://doi.org/10.1093/humrep/dew246.
3. Yakubu, M.T.; Akanji, M.A. Effects of aqueous extract of *Massularia acuminate* stem on the sexual behaviour of male Wistar rats. Evid-Bas. Complement. Alter. Med. 2011, 10. https://doi.org/10.1155/2011/738103.
4. Akre, C.; Berchtold, A.; Gmel, G.; Suris, J.C. The evolution of sexual dysfunction in young men aged 18-25 years. The J Adolesc Health: official publication of the Society for Adolescent Medicine, 2014, 55(6), 736–743. https://doi.org/10.1016/j.jadohealth.2014.05.014.
5. Kumar, N.; Singh, A.K. Trends of male factor infertility, an important cause of infertility: a review of the literature. J. Hum. Reprod. Sci. 2015, 8, 191–196. https://doi.org/10.4103/0974-1208.170370.
6. Mascarenhas, M.N.; Flaxman, S.R.; Boerma, T.; Vanderpoel, S.; Stevens, G.A. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLOS Med, 2012, 9(12), e1001356. https://doi.org/10.1371/journal.pmed.1001356.
7. Bella, A.J.; Lee, J.C.; Carrier, S.; Bénard, F.; Brock, G.B. CUA Practice guidelines for erectile dysfunction. Can. Urol. Assoc. J. 2015, 9(1-2), 23-9. https://doi.org/10.5489/cuaj.2699.
8. Giugliano, F.; Maiorino, M.; Bellastella, G.; Gicchino, M.; Giugliano, D.; & Esposito, K. Determinants of erectile dysfunction in type 2 diabetes. Int. J. Imp. Res. 2010, 22(3), 204–209. https://doi.org/10.1038/ijir.2010.1.
9. Thorve, V. S.; Kshirsagar, A.D.; Vyawahare, N. S.; Joshi, V.S.; Ingale, K. G.; Mohite, R.J. Diabetes-induced erectile dysfunction: epidemiology, pathophysiology and management. J. Diabet. Complicat. 2011, 25(2), 129–136. https://doi.org/10.1016/j.jdiacomp.2010.03.003.
10. Tamás, V.; Kempler, P. Sexual dysfunction in diabetes. Handbook of Clinical Neurology 2014, 126, 223–232. https://doi.org/10.1016/B978-0-444-53480-4.00017-5.
11. Colson, M.H. Sexual dysfunction and chronic illness. Part 1. Epidemiology, impact, and significance. Sexologies. 2016, 25(1), 5–11. https://doi.org/10.1016/j.sexol.2016.01.007.
12. Maiorino, M.I; Bellastella, G.; Esposito, K. Diabetes and sexual dysfunction: current perspectives. Diabet. Metab. Syndr. Obes. 2014, 7, 95-105. https://doi.org/10.2147/DMSO.S36455.
13. Huang, K.; Yin, S.; Xiao, Y.; Wang, J.; Cui, J.; Wang, J.; Bai, Y. Sexual dysfunction in patients with diabetes: association between remnant cholesterol and erectile dysfunction. Lipid Health Dis. 2024, 23, 55. https://doi.org/10.1186/s/2944-024-02046-8.
14. Esposito, K.; Giugliano, D. Obesity, the metabolic syndrome, and sexual dysfunction in men. Clin. Pharmacol. Ther. 2011, 90(1), 169-173. https://doi.org/10.1038/clpt.2011.91.
15. Ismail, E.A.; Younis, S.E.; Ismail. I.Y.; El-Wazir, Y.M.; El-Sakka, A.I. Early administration of phosphodiesterase 5 inhibitors after induction of diabetes in a rat model may prevent erectile dysfunction. Andrology. 2020, 8(1), 241-248. https://doi.org/10.1111/andr.12668.
16. Minas, A.; Fernandes, A.C.C.; Maciel Júnior, V.L.; Adami, L.; Intasqui, P.; Bertolla, R.P. Influence of physical activity on male fertility. Andrologia. 2022,

https://doi.org/10.56717/jpp.2024.v03i01.025

54(7), e14433. https://doi.org/10.1111/and.14433.

17. Brant, W.O.; Bella, A.J.; Lue, T.F. Treatment options for erectile dysfunction. Endocrinolog. Metabol. Clin. North Am. 2007, 36(2), 465–479. https://doi.org/10.1016/j.ecl.2007.02.001.
18. Dean, R.C.; Lue, T.F. Physiology of penile erection and pathophysiology of erectile dysfunction. Urolog. Clin. North Am. 2005, 32(4), 379–v. https://doi.org/10.1016/j.ucl.2005.08.007.
19. Esposito, K.; Giugliano, D. Obesity, the metabolic syndrome, and sexual dysfunction in men. Clin Pharmacol. Ther. 2011, 90(1), 169-173. https://doi.org/10.1038/clpt.2011.91.
20. Nehra, A.; Jackson, G.; Miner, M.; Billups, K.L.; Burnett, A.L.; Buvat, J.; Carson, C.C.; Cunningham, G.R.; Ganz, P.; Goldstein, I.; Guay, A.T.; Hackett, G.; Kloner, R.A.; Kostis, J.; Montorsi, P.; Ramsey, M.; Rosen, R.; Sadovsky, R.; Seftel, A.D.; Shabsigh, R.; Vlachopoulos, C.; Wu, F.C. The Princeton III Consensus recommendations for the management of erectile dysfunction and cardiovascular disease. Mayo Clin. Proc. 2012, 87(8), 766-78. https://doi.org/10.1016/j.mayocp.2012.06.015.
21. Oguntibeju, O.O.; Aboua, Y.; Kachepe, P. Possible therapeutic effects of vindoline on testicular and epididymal function in diabetes-induced oxidative stress male Wistar rats. Heliyon. 2020, 6(4), e03817. https://doi.org/10.1016/j.heliyon.2020.e03817.
22. Ngaha, N.M.I.; Dahlan, I.; Massoma, L.D. *Alchornea cordifolia,* a special plant for traditional medicine: A review. J. Agroecolog. Nat. Res. Manage. 2016, 3(2), 140-144.
23. Noundou, X. Isolation and identification of anti-cancer compounds from Alchornea species and their encapsulation in nanostructure drug delivery system. The Ph.D. Thesis University of Johannesburg. 2012; received from htt://ujdigispace.Uj.ac.za.
24. Adeshina, G.; Kunle, O.; Onaolapo, J.; Ehinmidu, J.; Odama, L. Phytochemical and antibacterial studies of the hexane extract of *Alchornea cordifolia* leaf, phytochemicals as Nutraceuticals-Global Approaches to their role in nutrition and Health**.** 2012, 65, 89-96.
25. Ngaha-Njile, M.I.; Lembe, D.M.; Koloko, B.L.; Meng, G.Y.; Ebrahimi, M.; Awad, E.A.; Baiee, F.H.; Kenmogne, H.; Hambe, M.; Mandengue, S.H. Sperm parameters quality and reproductive effects of methanolic extract of *Alchornea cordifolia* leave on senescent male rats. Andrologia 2019, 00:e13359, https://doi.org/10.1111/and.13359
26. Ejeh, S.A.; Abu, H.A.; Onyeyili, P.A.; Abenga, J.N.; Ogbe, R.J.; Abalaka, S.E. Phytochemical, metal analysis, and in-vitro antioxidant activity of ethanol leaf extract of *Alchornea cordifolia* (Schumach and Thonn) Mùll Arg (Euphorbiaceae). Trop. J. Nat. Prod. Res. 2023, 7(9), 4070-4075. https://doi.org/10.26538/tjnpr/v7i9.34.
27. McMillin, J.M. Blood Glucose. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths; 1990. Chapter 141.
28. Mos, J.; Mollet, I.; Tolboom, J.T.; Waldinger, M.D.; Olivier, B. A comparison of the effects of different serotonin reuptake blockers on sexual behaviour of the male rat. Eur Neuropsychopharmacol. 1999, 9(1-2), 123-135. https://doi.org/10.1016/s0924-977x (98)00015-7.
29. Öztaş, E.; Yılmaz, T.E.; Güzel, E.; Sezer, Z.; Okyar, A.; Özhan, G. Gliclazide alone or in combination with atorvastatin ameliorated reproductive damage in streptozotocin-induced type 2 diabetic male rats. Saudi Pharm. J. 2019, 27(3), 422-431. https://doi.org/10.1016/j.jsps.2019.01.003.
30. Melendez-Ramirez, L.Y.; Richards, R.J.; Cefalu, W.T. Complications of type 1 diabetes. Endocrin. Metabol. Clin. North Am. 2010, 39(3), 625–640. https://doi.org/10.1016/j.ecl.2010.05.009.
31. Ding, G.L.; Liu, Y.; Liu, M.E. et al. The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. Asian J. Androl. 2015, 17(6), 948-953. https://doi.org/10.4103/1008-682X.150844.
32. Mohammed, R.K.; Ibrahim, S.; Atawodi, S.E.; Eze, E.D.; Suleiman, J.B.; Ugwu, M.N.; & Malgwi, I.S. Antidiabetic and haematological effects of n-butanol fraction of *Alchornea cordifolia* leaf extract in streptozotocin-induced diabetic Wistar rats. Sci. J. Biol. Sci. 2(3), 45-53. https://www.europub.co.uk/articles/-A-1080.
33. Okoro, I.O; Okoro, E.O.; Isoje, F.E. In vitro, antidiabetic and antioxidant activities of *Alchornea cordifolia* 394 stem bark. Nig. J. Pure. Appl. Sci. 2022, 35(1), 4322-4332. https://doi.org/10.48198//njpas/22.a23.
34. Bhowmik, B.; Afsana, F.; Ahmed, T.; Akhter, S.; Choudhury, H. A.; Rahman, A.; Ahmed, T.; Mahtab, H.; Khan, A.K. Obesity and associated type 2 diabetes and hypertension in factory workers of Bangladesh. BMC Research Notes, 2015, 8, 460. https://doi.org/10.1186/s13104-015-1377-4.
35. Yusuf, S.; Rangarajan, S.; Teo, K.; Islam, S.; Li, W.; Liu, L.; Bo, J.; Lou, Q.; Lu, F.; Liu, T.; Yu, L.; Zhang, S.; Mony, P.; Swaminathan, S.; Mohan, V.; Gupta, R.; Kumar, R.; Vijayakumar, K.; Lear, S.; Anand, S. PURE Investigators. Cardiovascular risk and events in 17 low-, middle-, and high-income countries. The N. Engl. J. Med, 2014, 371(9), 818–827. https://doi.org/10.1056/NEJMoa1311890.
36. Crocetto, F.; Barone, B.; Manfredi, C.; Trama, F.; Romano, L.; Romeo, M.; Russo, G.; Sicignano, E.;

https://doi.org/10.56717/jpp.2024.v03i01.025

Persico, F.; Aveta, A.; Spirito, L.; Napolitano, L.; Imbimbo, C.; & Tarantino, G. Are insulin resistance and non-alcoholic fatty liver disease associated with Peyronie's disease? A pilot study. J. Physiol. Pharmacol. 2022, 73(1), https://doi.org/10.26402/jpp.2022.1.05.

37. Gazzaruso, C.; Coppola, A.; Giustina, A. Erectile dysfunction and coronary artery disease in patients with diabetes. Curr. Diab. Rev. 2011, 7(2), 143–147. https://doi.org/10.2174/157339911794940693.
38. Mallidis, C.; Agbaje, I.; McClure, N.; Kliesch, S. Einfluss des Diabetes mellitus auf die Fertilität des Mannes: Ein wenig untersuchter Faktor der männlichen Infertilität [The influence of diabetes mellitus on male reproductive function: a poorly investigated aspect of male infertility]. Der Urologe. Ausg. A 2011, 50(1), 33–37. https://doi.org/10.1007/s00120-010-2440-3.
39. Ejeh, S.A.; Abalaka, S.E.; Usende, I.L.; Alimi, Y.A.; Oyelowo, F.O. Acute toxicity, oxidative stress response and clinicopathological changes in Wistar rats exposed to aqueous extract of Uvaria chamae leaves. Sci. Afr. 2019, 3(1),1-9. https://doi.org/10.1016/j.sciaf.2019.e00068.
40. Njila, M.I.N.; Meng, G.Y.; Ebrahimi M.; Awad, E.A.; Baiee, F.H.; Lembe, D.M.; Kenmogne, H.; Koloko, B.L.; Hambe, M.; Mandengue, S.H. Effects of methanolic extract of *Alchornea cordifolia* leaves on the sexual behaviour of senescent and sexually inexperienced rats. J. Phytopharmacol. 2018, 7(6), 471-476.
41. Dasofunjo, K.; Asuk, A.A.; Ezugwu, H.C.; Nwodo, O.F.C.; Olatunji, T.L. Aphrodisiac effects of ethanol extract of *Piliostigma thonningii* leaf on male Albino Wistar rats. J. Appl. Pharm. Sci. 2013, 3(10), 130-135. https://doi.org/10.7324/JAPS.2013.31023.
42. Fouche, G.; Afolayan, A. J.; Wintola, O. A.; Khorombi, T. E.; Senabe, J. Effect of the aqueous extract of the aerial parts of Monsonia angustifolia E. Mey. Ex A. Rich., on the sexual behaviour of male Wistar rats. BMC Complement. Altern. Med. 2015, 15, 343. https://doi.org/10.1186/s12906-015-0880-4.
43. Nantia, E.A.; Moundipa, P.F.; Monsees, T.K.; Carreau, S. Medicinal plants as a potential male antifertility agent: A review. Basic Clin. Androl. 2009, 19, 148–158. https://doi.org/10.1007/s12610-009-0030-2.
44. Boublata, N.I.; Saadane, F.Z.; Habbachi, S.; Bouzar, A.; Habbachi, W.; Benhissen, S. Effects of *Cleome arabica* ethanolic extract on Wistar rat behaviour, Biochemical parameters, and ACTH hormone. Biosci. Res. 2021, 18, 1471-1479. https://doi.org/10.31893/jabb.21035.
45. Yoshitake, T.; Yoshitake, S.; Kehr. J. The Ginkgo biloba extract EGb 761(R) and its main constituent flavonoids and ginkgolides increase extracellular dopamine levels in the rat prefrontal cortex. British J. Pharmacol. 2010, 159(3), 659–668. https://doi.org/10.1111/j.1476-5381.2009.00580.x
46. Zhou, F.; Li, G. Y.; Gao, Z. Z.; Liu, J.; Liu, T.; Li, W. R.; Cui, W. S.; Bai, G. Y.; & Xin, Z. C. The TGF-β1/Smad/CTGF pathway and corpus cavernosum fibrous-muscular alterations in rats with streptozotocin-induced diabetes. J. Androlog. 2012, 33(4), 651–659. https://doi.org/10.2164/jandrol.111.014456.
47. Pontes, D.A.; Fernandes, G.S.; Piffer, R.C.; Gerardin, D.C.; Pereira, O.C.; Kempinas, W.G. Ejaculatory dysfunction in streptozotocin-induced diabetic rats: the role of testosterone. Pharmacol. Rep. PR. 2011, 63(1), 130–138. https://doi.org/10.1016/s1734-1140(11)70407-8.
48. Arafa, M.; El Tabie, O. Medical treatment of retrograde ejaculation in diabetic patients: a hope for spontaneous pregnancy. J. Sex. Med. 2008, 5(1), 194–198. https://doi.org/10.1111/j.1743-6109.2007.00456.x
49. Wani, J.A.; Achur, R.N.; Nema, R.K. Phytochemical screening and aphrodisiac activity of *Asparagus racemosus.* Int. J. Pharm. Sci. Res. 2011, 3(2), 112-115.
50. Malviya, N.; Jain, S.; Gupta, V.B.; Vyas, S. Recent studies on aphrodisiac herbs for the management of male sexual dysfunction: A review. Acta Pol. Pharm. 2011, 68(1), 3-8.
51. Pareek, H.; Sharma, S.; Jain, G. Evaluation of ameliorative efficacy of *Tridax procumbens* on the sexual performance of male rats with alloxan-induced diabetes. 2014, 3. 2319-389150.
52. Preedapirom, W.; Changwichit, K.; Srisawang, P.; Ingkaninan, K.; and Taepavarapruk, P. Aphrodisiac activity of *Eulophia macrobulbon* extract on erectile dysfunction in male aged rats. BioMed. Res. Int. 2018, 6217029. https://doi.org/10.1155/2018/6217029.

https://doi.org/10.56717/jpp.2024.v03i01.025