Comparative Clinical Pathology (2022) 31:397–407
https://doi.org/10.1007/s00580-022-03339-z

ORIGINAL ARTICLE

Alkaloids-rich extracts from *Cannabis sativa*, *Datura stramonium*, and *Nicotiana tabacum* modulate sexual behavior and key enzymes relevant to sexual function in rats

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Received: 28 September 2021 / Accepted: 9 March 2022 / Published online: 23 March 2022

Abstract
Datura stramonium and *Nicotiana tabacum* are plant-based psychoactive substances commonly employed as alternatives to the illicit plant, *Cannabis sativa*, during aphrodisiac concoction preparation. The present study compared the effect of illicit (*Cannabis sativa*) and licit (*Nicotiana tabacum* and *Datura stramonium*) psychoactive substances on sexual behavior and several biochemical parameters in the penile tissue of male rats. Alkaloid extracts were orally administered in doses of 5, 50, 500, and 2000 mg/kg bwt for 90 days. On day 90, behavioral studies were carried out, rats were sacrificed, penile tissues removed, and tissue homogenate prepared. Penile tissue homogenates were assayed for phosphodiesterase-5 (PDE-5), arginase, acetylcholinesterase, angiotensin-1 converting enzyme (ACE) and lactate dehydrogenase (LDH) activities, nitric oxide (NO), and reactive oxygen species (ROS) levels. Findings showed that alkaloid extracts of *Datura stramonium* reduced the mounting and intromission number, depleted the level of NO, increased the activities of PDE-5, arginase, ACE, and reduced AChE activities, while *Datura stramonium* and *Nicotiana tabacum* alkaloids increased ROS levels and inhibited the activity of LDH. In conclusion, the use of *N. tabacum* as an alternative to *C. sativa* during aphrodisiac concoction preparation may be justified while *D. stramonium* inclusion may not be to mediate aphrodisiac effects.

Keywords *Cannabis sativa* · *Datura stramonium* · *Nicotiana tabacum* · Sexual function · Sexual behavior

Introduction

Sexual function is a multifaceted action comprising of a complex interplay between the endocrine, vascular, and nervous systems as well as other biostructures that are essential for satisfactory sexual arousal, intercourse, and performance. Despite the importance of satisfactory sexual function to the normal male species, demanding living conditions, certain prescriptive and several nonprescriptive medications, change in lifestyle, pollution, dietary deficiencies, and toxicants have negative effects on sexual life and result in sexual dysfunction (Chen et al. 2019; Kenneth 2001), with World Health Organization (WHO) listing sexual dysfunction as a major public health priority in both the rich and poor economic nations (WHO 2016). This has triggered research to synthesize drugs, such as Alprostadil, Buspirone, Cyproheptadine, and Sildenafil, which have been implicated to have serious side effects (Malviya et al. 2016). In Nigeria, a variety of natural products comprising crude extracts and aphrodisiac concoctions from plants are employed as alternatives to the synthesized drugs to mediate sexual motivation, prolonged duration, improved desire, and alertness with most of the crude extracts and aphrodisiac concoctions made from psychoactive plants, such as *Cannabis sativa* (Bonini et al. 2018).

Due to *C. sativa* been illicit in Nigeria, naturally available and licit psychoactive plants, *Datura stramonium* and *Nicotiana tabacum* are employed as alternatives for its traditional medicine and recreational usage. Their rates of prevalence are on the increase due to the different and new experiences they offer (Abdurahman et al. 2019; Dumbili 2020). Although, the use of crude extracts and aphrodisiac concoctions from these psychoactive plants for treatment and management of sexual dysfunction have been observed in folklore. There has been dearth of scientific validation for the traditional claims of the plants to improve or deplete

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sexual behavior and function during chronic exposure. Furthermore, the comparative effects between the illicit and licit substances to mediate sexual function or dysfunction in chronic studies are scarce.

Sexual behavior and function are initiated via sexual excitement, a process implicated to elevate nitric oxide (NO) release from endothelial cells and nervous terminations via nitric oxide synthase (NOs) action. Nitric oxide synthase (NOs) uses molecular oxygen and L-arginine as the substrate to trigger the release of NO, which then diffuses across the localized cell membrane into smooth muscles to trigger guanylyl cyclase enzymatic activation. This process in turn catalyzes guanosine-5′-triphosphate (GTP) biotransformation into 3′-5′-cyclic guanosine monophosphate (cGMP), a second messenger. The production of cGMP results in cGMP-dependent protein kinase (PKG) step activation, which in turn phosphorylates proteins that are crucial for relaxation. Consecutively, cGMP production also triggers intracellular calcium ion (Ca^{2+}) concentrations reduction. Both post-cGMP production processes trigger arterial and trabecular smooth muscle relaxation, leading to arterial dilation, elevated blood flow to the penis, and a successive erection and stiffness of the penis (Masuku et al. 2020). However, inhibition feedback is induced by phosphodiesterase-5 (PDE-5) enzyme activity on the cGMP to induce penile detumescence and arteriolar vasoconstriction (Castela and Costa 2016), a process if overactivated may result in reduced cGMP levels and subsequently sexual dysfunction. Therefore, the ability of a drug to suppress PDE-5 enzyme activity will improve cGMP levels as well as sexual function (Odubanjo et al. 2018). More so, suppression of acetylcholinesterase (AChE) activity increases acetylcholine (ACh) concentrations at penis corpus cavernosum smooth muscle cells to induce relaxation at the muscle and increase penile tumescence by triggering endothelium NO production (Ojo et al. 2019). The renin-angiotensin system (RAS) has also been observed to play key roles in sexual function (Masuku et al. 2020). Meanwhile, reactive oxygen species (ROS) production has been implicated to oxidize tetrahydrobiopterin (BH_4) (a substrate for NO synthesis) to dihydrobiopterin (BH_2), resulting in uncoupling of NOs, which in turn results in superoxide production instead of NO production, thus leading to decreased NO levels (Zhao et al. 2015). Another essential toxicity indicator in sexual function/dysfunction is lactate dehydrogenase (LDH), an oxidoreductase (XOR) enzyme that is released into the bloodstream by cells after tissue damage (Akintunde et al. 2015). Several studies have evaluated the effects of the plants on sexual function but the present study will focus on comparing the abilities of the alkaloids-rich extracts from the plants to modulate sexual behavior and key enzymes relevant to sexual function in male Wister rats to justify their use as aphrodisiac agents.

Materials and methods

Sample collection and preparation

Cannabis sativa and *Nicotiana tabacum* leaves, and *Datura stramonium* seeds were obtained in towns of Akure South Local Government of Ondo State in Nigeria. The seeds of *D. stramonium* and leaves of *C. sativa* and *N. tabacum* were authenticated and identified at the CERAD (Centre for Research and Development) unit of the Federal University of Technology, Akure, Nigeria. The seeds and leaves were cleansed from debris, stalks, and other unwanted materials, and were afterward powdered with an electric blender.

Chemicals and reagents

Acetylthiocholine iodide, acetic acid, angiotensin-1 converting enzyme, methanol, estradiol benzoate, progesterone, hydrogen peroxide, L-arginine, Coomassie blue G, para-nitrophenyl phenylphosphonate (PNPPP), nitrate, and Ellman's reagent was obtained from Sigma Chemical Co, St. Louis, MO, USA; Organon Limited, Kolkata, India; and Cadilla Healthcare Limited, Daman, India. All reagents used were of analytical grade and the water used was distilled.

Preparation of alkaloid-rich extracts

Alkaloid-rich extracts were prepared of *Cannabis sativa* and *Nicotiana tabacum* leaves and *Datura stramonium* seeds according to the methods of Ademiluyi et al. (2016).

Experimental protocol

Sixty-five (65) Wistar albino rats (male) weighing an average of 203 ± 10 g were purchased from the Biochemistry department animal house in the Federal University of Technology, Akure, Nigeria. Their handling and use were approved by the Animal ethical committee, Centre for Research and Development (CERAD) of the Federal University of Technology, Akure with the ethical number FUTA/ETH/2020/016. Stainless steel cages were used to house the Wistar albino rats in a room where 12-h light/dark cycle, relative humidity (60–70%), and room temperature (25–27 °C) were maintained all through the timing of the experiment. The rats were acclimatized for 2 weeks, after which they were grouped with each group consisting of five (5) rats randomly selected and housed in a cage. The animals were grouped and doses of extract selected according to the Organization for Economic

Co-operation and Development (OECD) guideline (2001) as described by Patrick-Iwuanyanwu et al (2010) as follows:

Group 1 consisted of the normal control rats orally administered distilled water only.
Group 2 consisted of rats orally administered alkaloid-rich extracts of *C. sativa* once daily at doses of 5 mg/kg bwt.
Group 3 consisted of rats orally administered alkaloid-rich extracts of *C. sativa* once daily at doses of 50 mg/kg bwt.
Group 4 consisted of rats orally administered alkaloid-rich extracts of *C. sativa* once daily at doses of 500 mg/kg bwt.
Group 5 consisted of rats orally administered alkaloid-rich extracts of *C. sativa* once daily at doses of 2000 mg/kg bwt.
Group 6 consisted of rats orally administered alkaloid-rich extracts of *D. stramonium* once daily at doses of 5 mg/kg bwt.
Group 7 consisted of rats orally administered alkaloid-rich extracts of *D. stramonium* once daily at doses of 50 mg/kg bwt.
Group 8 consisted of rats orally administered alkaloid-rich extracts of *D. stramonium* once daily at doses of 500 mg/kg bwt.
Group 9 consisted of rats orally administered alkaloid-rich extracts of *D. stramonium* once daily at doses of 2000 mg/kg bwt.
Group 10 consisted of rats orally administered alkaloid-rich extracts of *N. tabacum* once daily at doses of 5 mg/kg bwt.
Group 11 consisted of rats orally administered alkaloid-rich extracts of *N. tabacum* once daily at doses of 50 mg/kg bwt.
Group 12 consisted of rats orally administered alkaloid-rich extracts of *N. tabacum* once daily at doses of 500 mg/kg bwt; and.
Group 13 consisted of rats orally administered alkaloid-rich extracts of *N. tabacum* once daily at doses of 2000 mg/kg bwt.

Experimental animals were administered the alkaloid-rich extracts through oral gavage within the hours of 09:00 to 11:00. They were allowed free access to rat chow and water ad libitum and the experiment lasted for 90 days after acclimatization.

Sexual behavioral assessment

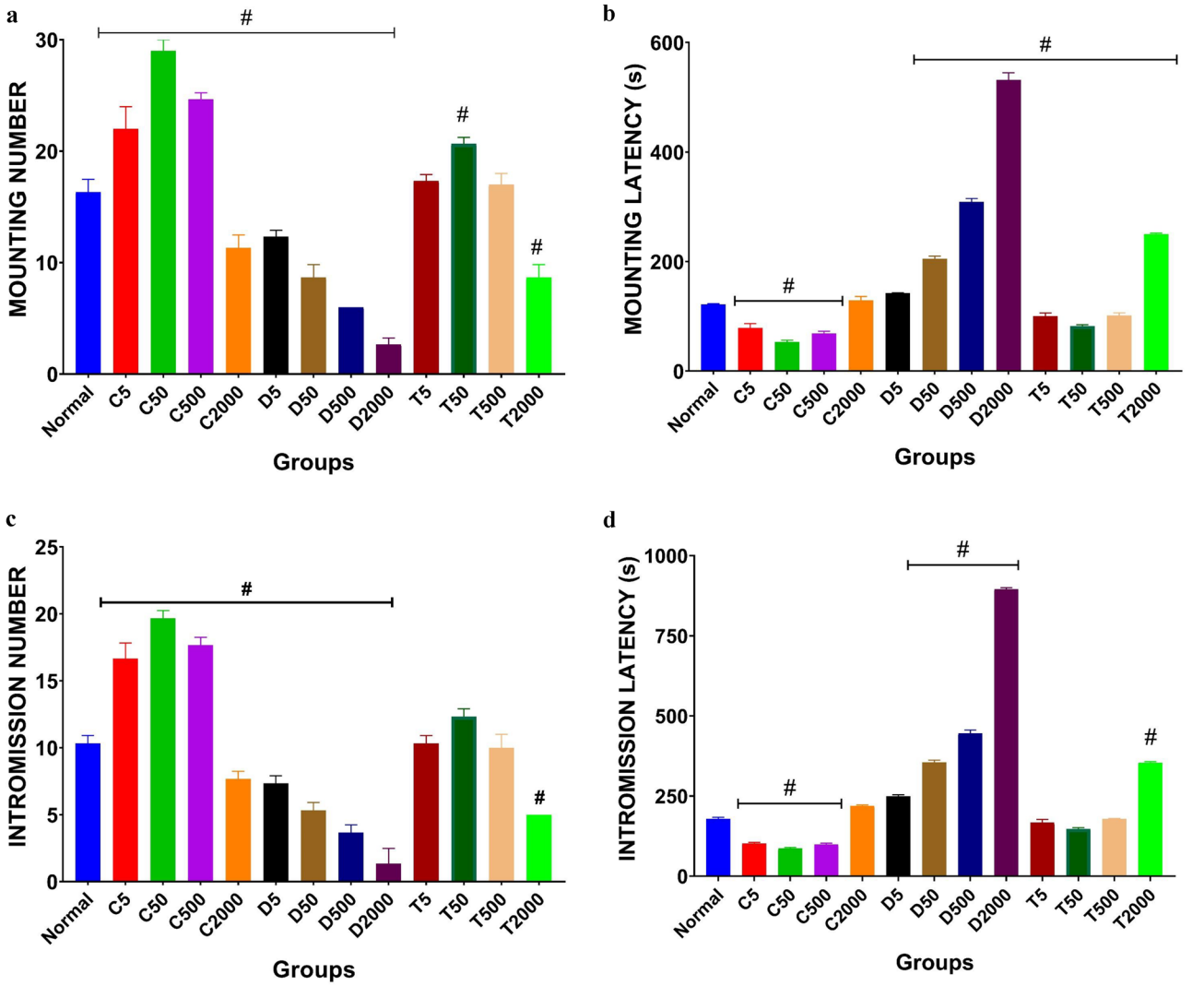
On the 90th day of administration, alkaloid-treated male rats were each paired with estrous Wistar albino female rats to assess the sexual behavior of the alkaloid-treated male rats. The Female rats were induced to estrous by the administration of estradiol benzoate at a dose of 2 µg/kg body weight at 48 h and progesterone, 500 µg/kg body weight at 4 h respectively before the commencement of sexual behavioral studies. The sexual behavior was assessed using a plastic box (80 × 60 × 60 cm) that is clear and appropriate for digital video recordings in a different room from the administration room for 30 min per session. The behavioral study was carried out during the early hours starting at 6:00 a.m. Parameters assessed for sexual behavior were mounting number and latency and intromission number and latency (Ademosun et al. 2019; Guohua et al. 2009).

Tissue homogenate preparation

On the 91st day after commencement of experiment, alkaloid-treated male rats were anesthetized using mild diethyl ether. Alkaloid-treated male rats were sacrificed while the penile tissues were excised and washed quickly in a cold saline solution, after which they were homogenized in 0.1 M physiological buffer (pH 7.4) and centrifuged in a 10,000 g refrigerated centrifuge for 10 min. The supernatant was decanted and used for biochemical analyses.

Biochemical assays

The total protein content of penile tissue homogenate was measured using Coomassie blue method with bovine serum albumin serving as the standard (Bradford 1976). Nitric oxide (NO) level was measured using 2% VCl3 in 5% HCl, 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride, and 2% sulfanilamide (in 5% HCl) while the reduction of nitrate to nitrite by VCl_3 was measured to determine the levels of NO at 540 nm (Miranda et al. 2001). The phosphodiesterase-5 activity was measured using *p*-nitrophenyl phenylphosphonate (5 mM) and 20 mM Tris buffer (pH 8.0), while the resultant *p*-nitrophenol produced was measured at 400 nm at intervals of 60, 120, 180, 240, and 300 s (Kelly and Butler 1977). Arginase activity was measured using 1.0 mM Tris–HCl buffer (pH) containing 1.0 mM $MnCl_2$ and 0.1 M arginine solution. The reaction was terminated with 2.5 ml Ehrlich reagent (2.0 g of *p*-dimethylaminobenzaldehyde in 20 ml of absolute HCl) after 10 min of incubation at 37 °C and measured at 450 nm (Zhang et al. 2001); acetylcholinesterase activity was measured using 5,5′-dithiobis (2-nitrobenzoic) acid (3.3 mM) prepared by 0.1 M buffer (pH 7.0) constituting of $NaHCO_3$. AChE iodide served as the substrate for the assay while absorbance was measured at 412-nm at intervals of 30, 60, 90, 120, 150, and 180 s (Perry et al. 2000). Angiotensin-1 converting enzyme (ACE) was measured using HHL (hippuryl-l-histidyl l-leucine) as substrate while the resultant hippuric acid (Bz-Gly) was measured to determine the activity



of ACE at 228 nm (Cushman and Cheung 1971). Reactive oxygen species (ROS) level was measured using *N–N*-diethyl-para-phenylenediamine (DEPPD) (6 mg/ml) and ferrous sulfate (4.37 µM) prepared in 0.1 M sodium acetate (pH 4.8). H_2O_2 production was used to measure ROS level at 505 nm (Hayashi et al. 2007). Lactate dehydrogenase activity was measured using a commercially available kit (Randox Laboratories UK) (Weisshaar 1975).

Statistical analysis

All data were analyzed GraphPad Prism version 8.0 for windows using one-way analysis of variance (ANOVA) followed by Duncan multiple tests. Data were expressed as mean ± standard error of mean (SEM) of five (5) rats per group.

Results

Figure 1 revealed the effects of alkaloid extracts of *Cannabis sativa*, *Datura stramonium*, and *Nicotiana tabacum* at varying doses (5, 50, 500, and 2000 mg/kg bwt) on the mounting number (1a), mounting latency (1b), intromission number (1c), and intromission latency (1d) in comparison to the normal control group. Typically, the administration of *C. sativa* significantly increased ($p < 0.05$) the mounting and intromission number at doses of 5 and 50 mg/kg bwt though a significant decline in mounting and intromission number was observed at higher doses of 500 and 2000 mg/kg bwt of *C. sativa*. Although similar trends were also observed with the alkaloid extracts of *N. tabacum* administration, the effects were not significantly different ($p < 0.05$) compared to the normal control group. However, results revealed that alkaloid extracts

◂**Fig. 1** **a** Mounting number. C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group. **b** C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group. **c** C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group. **d** C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group

of *D. stramonium* significantly reduced ($p < 0.05$) the mounting and intromission number in a concentration-dependent manner compared to the normal control group. Furthermore, the effects of alkaloid extract of *C. sativa* and *N. tabacum* on mounting and intromission were observed to be biphasic while alkaloid extracts of *D. stramonium* were observed to significantly increase ($p < 0.05$) the mounting and intromission latency in a concentration-dependent manner compared to the normal control group.

The result of the effects of *C. sativa*, *D. stramonium*, and *N. tabacum* at varying doses (5, 50, 500, and 2000 mg/kg bwt) on nitric oxide (NO) levels in normal rats is presented in Fig. 2. Results revealed that administration of *C. sativa* alkaloids at 5, 50, and 500 mg/kg bwt significantly ($p < 0.05$) elevated the NO level in comparison with the normal control group. The most prominent effect of *C. sativa* was observed at 500 mg/kg bwt while a decline in trend was observed at 2000 mg/kg bwt *C. sativa* alkaloid administration. Additionally, a similar trend was observed with *N. tabacum* alkaloid administration, with the effects of *C. sativa* alkaloids posing a prominent NO level than that observed with *N. tabacum* alkaloids. However, *D. stramonium* alkaloids administration significantly ($p < 0.05$) depleted the level of NO in a concentration-dependent manner, when compared to the normal control group.

Figure 3 revealed the effects of *C. sativa*, *D. stramonium*, and *N. tabacum* at varying doses (5, 50, 500, and 2000 mg/kg bwt) on the activity of phosphodiesterase-5 (PDE-5) in normal rats. *C. sativa* alkaloids significantly reduced ($p < 0.05$) the activities of PDE-5 in normal rats when compared to the normal control group. Meanwhile, *D. stramonium* alkaloids significantly ($p < 0.05$) increased the activities of PDE-5 in a concentration-dependent manner. However, reduction in PDE-5 activities was also observed with *N. tabacum* alkaloid administration while showing no significant level of difference ($p < 0.05$) in comparison to the normal control group.

C. sativa, *D. stramonium*, and *N. tabacum* at varying doses (5, 50, 500, and 2000 mg/kg bwt) also caused significant ($p < 0.05$) effects on arginase activity in normal rats (Fig. 4). The activities of arginase were significantly reduced ($p < 0.05$) in *C. sativa* and *N. tabacum* alkaloids treated groups in a concentration-dependent manner in comparison to the normal control group. Whereas, administration of *D. stramonium* alkaloid caused a significant ($p < 0.05$) increase in the activities of arginase in a concentration-dependent manner in comparison to the normal control group.

The result of *C. sativa*, *D. stramonium*, and *N. tabacum* administration at varying doses (5, 50, 500, and 2000 mg/kg bwt) on acetylcholinesterase (AChE) activity are presented in Fig. 5. The activity of AChE was significantly ($p < 0.05$) reduced in normal rats at *C. sativa*, *D. stramonium*, and *N. tabacum* administration in a concentration-dependent manner, with *D. stramonium* alkaloid posing the most prominent effect when compared to the normal control group.

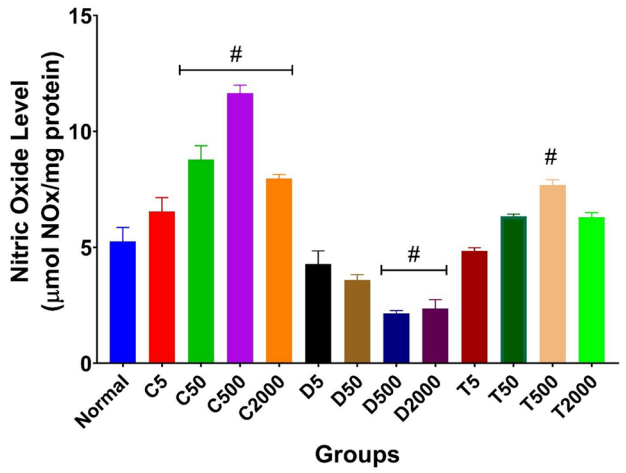


Fig. 2 Nitric oxide level. C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group

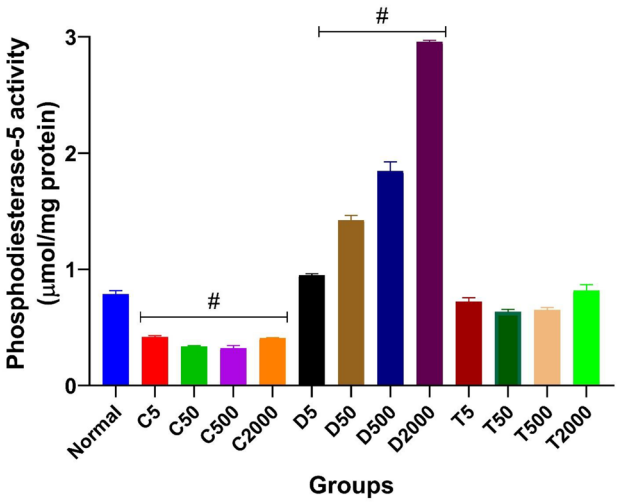


Fig. 3 Phosphodiesterase-5 activity. C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group

Figure 6 showed that the activities of angiotensin-1 converting enzyme (ACE) were significantly ($p < 0.05$) reduced in a concentration-dependent manner during chronic administration of *C. sativa* and *N. tabacum* at doses of 5, 50, and 500 mg/kg. However, the reduction pattern was absent at 2000 mg/kg bwt administration which showed a concomitant increase but lower than that observed in the normal control group. Interestingly, chronic administration of *D. stramonium* was observed to increase ACE activities in a concentration-dependent manner.

Figure 7 reveals the effects of *C. sativa*, *D. stramonium*, and *N. tabacum* at varying doses (5, 50, 500, and 2000 mg/kg bwt) on reactive oxygen species (ROS) levels in comparison with normal control rats. Administration of *C. sativa* alkaloid at 5 and 50 mg/kg bwt was observed to lower the level of ROS while higher doses of 500 and 2000 mg/kg bwt of the alkaloids significantly ($p < 0.05$) elevated ROS level. However, *D. stramonium* and *N. tabacum* alkaloids administration significantly ($p < 0.05$) increased ROS level in normal rats, with *D. stramonium* posing a higher level of ROS production.

The effects of *C. sativa*, *D. stramonium*, and *N. tabacum* at varying doses (5, 50, 500, and 2000 mg/kg bwt) on the activity of lactate dehydrogenase (LDH), a functional asthenospermia marker in rat testes, are shown in Fig. 8. Administration of *C. sativa* alkaloids improved the activity of LDH at 5 and 50 mg/kg bwt doses, though dosages of 500 and 2000 mg/kg bwt showed inhibitory effects on LDH activities. Effects of *D. stramonium* and *N. tabacum* were revealed to inhibit significantly ($p < 0.05$) the activities of LDH. The figure further revealed that the potency of *D. stramonium* alkaloids to inhibit LDH activities was higher in comparison to that expressed by *N. tabacum* and alkaloids.

Discussions

As observed in the present study, mounting number, mounting latency, intromission number, and intromission latency were greatly altered by exposure to the different concentrations of *C. sativa*, *D. stramonium*, and *N. tabacum* alkaloids

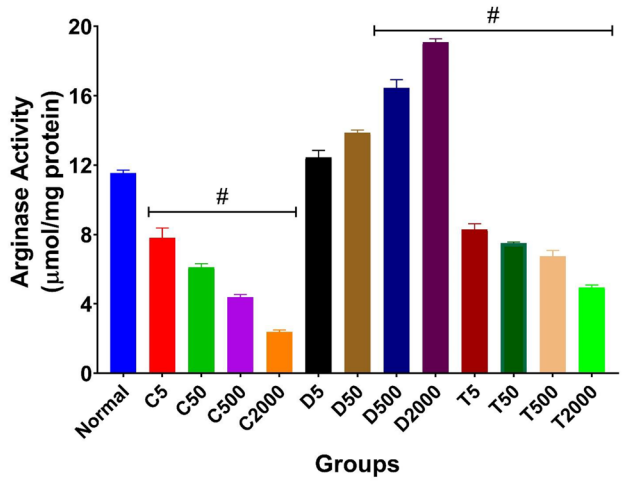


Fig. 4 Arginase activity. C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group

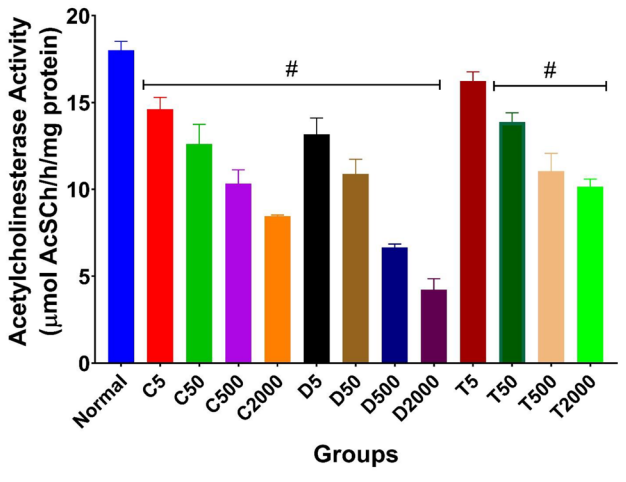


Fig. 5 Acetylcholinesterase activity. C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group

in comparison to the normal control group. Interestingly, the study revealed a curvilinear model of effect on sexual activities. This biphasic model of the effect of the alkaloids is consistent with other studies that observed that chronic administration of plant-based psychoactive substances improves sexual function at low doses while at high doses impairment of testosterone secretion and sperm production was observed (German et al. 2014; Johnson et al. 2020). At the lower doses of administration (5 and 50 mg/kg bwt), the alkaloid extracts tend to favorably influence sexual activities but at higher doses of 500 and 2000 mg/kg bwt, the sexual activities were adversely affected with *C. sativa* having the least deterioration. The decreased mounting and intromission latencies observed at lower doses may indicate the potential of *C. sativa* and *N. tabacum* to enhance the sex desire and sexual behavior in sexual dysfunctional individuals (Outhoff 2009). The trend of compromised sexual behavior at higher doses of *C. sativa* and *N. tabacum* and decreased sexual activities in *D. stramonium* groups as observed in the present study could result from elevated arginase and PDE-5 activities which have been established to result in lower copulation and sexual dysfunction (Adefegha et al. 2021).

Nitric oxide (NO) release is central to the initiation of activation of soluble guanylate cyclase, inhibition of vascular smooth muscle proliferation, smooth muscle cell relaxation, and maintenance of vascular endothelial cells, which leads to vasodilation and reduced intracellular calcium levels during sexual activities (Andersson 2011). Results from the present study showed that *C. sativa* and *N. tabacum* posed a significant curvilinear effect while *D. stramonium* showed a concentration-dependent reduction in NO level. The possible explanation may be that the *C. sativa* and *N. tabacum* alkaloids increased endothelial tissue NOs activity expression and/or inhibited NO elimination, thereby resulting in increased nitric oxide bioavailability (Sasatomi et al. 2008).

The first-line of drugs endorsed by the United States of Food and Drug Administration (FDA) in the management and treatment of sexual dysfunction are drugs that can prevent the PDE-5 enzyme from depleting cGMP levels and impeding arterial blood flow, a process that results in

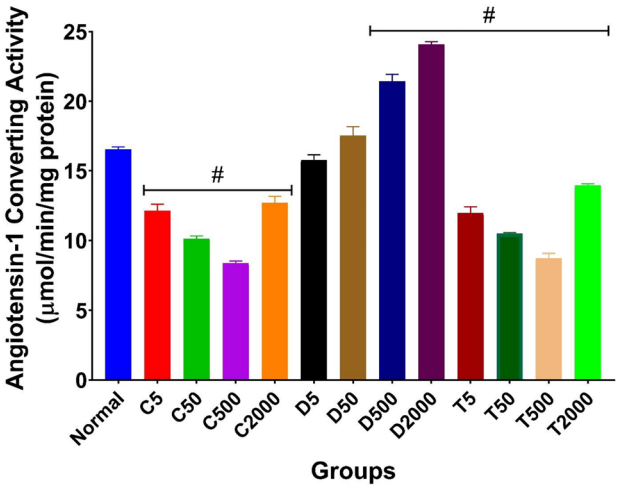


Fig. 6 Angiotensin-1 converting enzyme activity. C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group

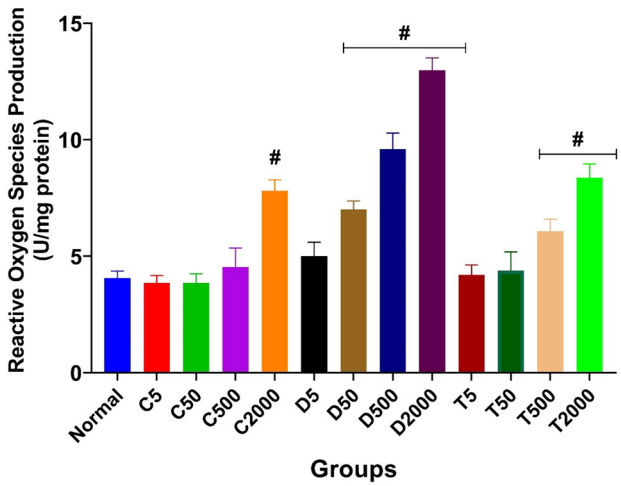


Fig. 7 Reactive oxygen species production level. C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group

vasodilation and corpus cavernosum tissue relaxation as well as prolonged penile erection and improved sexual function (Hatzimouratidis et al. 2016; Sommer and Schulze 2005). Interestingly, *C. sativa* and *N. tabacum* alkaloids inhibited the activities of PDE-5 while *D. stramonium* alkaloids upregulated the activities of the enzyme. The ability of *C. sativa* and *N. tabacum* alkaloids to inhibit PDE-5 may be one of the mechanisms employed by the plant alkaloids to improve mounting and intromission numbers as observed in the present study, as the inhibition of PDE-5 will reduce rapid hydrolysis of cGMP by PDE-5 and thereby, prolonged penile erection and improved sexual functions. Meanwhile, the dose-dependent increase in PDE-5 activity observed with *D. stramonium* administration may be due to disorder in the NO/cGMP pathway, a major factor in sexual dysfunction (Lugnier 2006).

Another key mediator of reduced NO production during sexual activity is elevated arginase activity as observed in the present study. Whenever the activity of arginase is elevated, hydrolysis of L-arginine to urea and ornithine is increased via urea cycle, while the bioavailability and concentration of NO are depleted, due to NO synthase and arginase dependence on the same substrate (L-arginine) (Caldwell et al. 2018). The present study observed that *C. sativa* and *N. tabacum* both decreased arginase activity significantly while *D. stramonium* increased arginase activity significantly. This may be the possible explanation for the effect of the alkaloids on NO release, as NO release is linked with an agent inhibitory effect on arginase activity (Lorenzen et al. 2009; Adefegha et al. 2018). Although most sexual dysfunction management/treatment drugs are PDE-5 inhibitors and not arginase inhibitors, administration of PDE-5 inhibitors to manage/treat sexual dysfunction where arginase activity prevails is usually ineffective. Therefore, the inhibitory effect of both *C. sativa* and *N. tabacum* alkaloid extracts on arginase activity may be another mechanism employed by the plants to improve the mounting and intromission number observed in the present study and further indicate their potential to improve sexual function as observed in folklore.

Acetylcholine (ACh), a parasympathetic neurotransmitter, mediates sexual function by relaxing the corpus cavernosum smooth muscle of the penis and enhances penile tumescence by triggering endothelial NO production (Ojo et al. 2019). Therefore, the ability of a drug to inhibit the

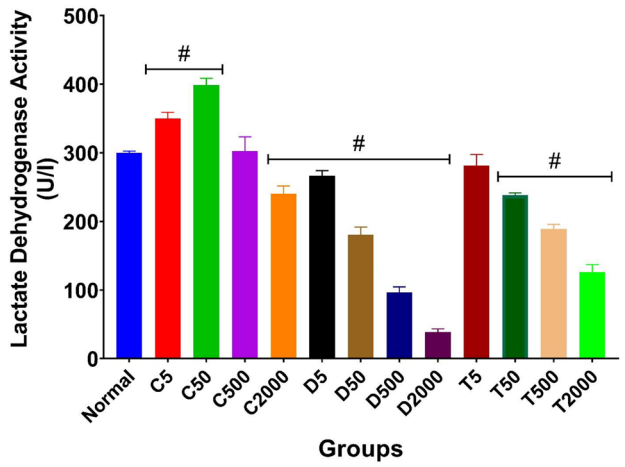


Fig. 8 Lactate dehydrogenase activity. C5—orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; C50—orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; C500—orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; C5000—orally administered alkaloid extracts of *C. sativa* at 5000 mg/kg bwt; D5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; D50—orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; D500—orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; D5000—orally administered alkaloid extracts of *D. stramonium* at 5000 mg/kg bwt; T5—orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; T50—orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; T500—orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; T5000—orally administered alkaloid extracts of *N. tabacum* at 5000 mg/kg bwt. Results are expressed as mean ± SEM ($n = 5$). Bars with superscript # are significantly ($p < 0.05$) from the normal control group

activity of AChE, the enzyme that hydrolysis ACh to choline and acetate, will increase ACh levels and enhance penile erection (Heidelbaugh 2010; Ojo et al. 2019). Interestingly, all the alkaloid extracts inhibited AChE activity significantly with *D. stramonium* showing the highest level of inhibition. This substantiates a previous study that implicated *D. stramonium* alkaloids (atropine, hyoscyamine, and scopolamine) as a potent cholinesterase inhibitor (Fasakin et al. 2021).

Another pathway that has also been implicated in sexual function/dysfunction pathophysiology is the renin-angiotensin system (RAS) pathway (Odubanjo et al. 2018). The enzyme angiotensin-1 converting enzyme (ACE) is the enzyme tasked with catalyzing angiotensin-1 conversion to the potent angiotensin-2, the main constituent of the renin-angiotensin system (RAS), which mediates stimulated contraction of the corpus cavernosum smooth muscles during sexual functions (Fraga-Silva et al. 2013; Oboh et al. 2017). Therefore, the high activities of ACE observed with *D. stramonium* administration may result in chronic high levels of angiotensin-2 which have been implicated to result in angiotensin type-1 receptor (AT_1R) activation, leading to stimulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and vasoconstriction, which results into excessive superoxide anions generation, depleted endothelial nitric oxide concentration, and sexual dysfunction (Ademiluyi et al. 2019; Jiménez et al. 2019; Passaglia et al. 2015; Rincón et al. 2015). However, the reduction in ACE activities observed during *C. sativa* and *N. tabacum* administration may suggest their ability to act as ACE inhibitors at the dosages.

Furthermore, the present study observed that the alkaloid extracts elevated ROS production in a concentration-dependent manner, with significant levels of increase observed mostly at 500 and 2000 mg/kg bwt. The study also observed that *D. stramonium* had the highest level of ROS production. The elevation of ROS level has been linked with angiotensin-2 production (Adefegha et al. 2018), a process that was also observed in the present study. Meanwhile, elevated ROS have been implicated to react with NO to produce peroxynitrite ($ONOO^-$) which decreases the bioavailability and concentration of NO (Zhao et al. 2015). Furthermore, the resulting peroxynitrite was implicated to cause the generation of powerful oxidants such as nitrogen dioxide (NO_2) and hydroxyl radicals which result in oxidative stress and consecutively, sexual dysfunction (Tabit et al. 2010).

LDH activity has been widely used in assessing cellular ATP levels during anaerobic conditions due to its stability (Akintunde et al. 2015). The present study observed that *C. sativa* had a curvilinear effect on the enzyme while *D. stramonium* and *N. tabacum* significantly reduced the enzyme activity. The inhibitory effect of *D. stramonium* and *N. tabacum*, and *C. sativa* only at high doses on LDH activity can indicate an alteration to the metabolic pathway responsible for energy (ATP) production. The finding agrees with previous studies that observed abnormal spermatogenesis and sexual dysfunction in link with depleted ATP levels (Klaassen and Watkins 2003; Akintunde et al. 2015), an indication that only *C. sativa* alkaloids at doses of 5 and 50 mg/kg bwt may have potential to ameliorate depleted ATP levels in sexual dysfunctional individuals.

Conclusions

Although, the relationship between psychoactive plant alkaloids and sexual function may be complex, the effect of the alkaloids on sexual behavior and key enzymes relevant to sexual function may proffer us mechanisms of action to justify their use in folklore as crude extracts or ingredients of aphrodisiac concoctions. According to our results, *C. sativa* and *N. tabacum* showed significant potency to improve sexual function (especially at 5 and 50 mg/kg bwt) while *D. stramonium* showed significant potency to facilitate sexual dysfunction across all doses during chronic exposure. This is an indication that the use of *D. stramonium* as ingredients of aphrodisiac concoctions may be to elevate the concoction's

psychoactive effects and not majorly to improve the aphrodisiac potentials of the concoction. However, the use of *C. sativa* and *N. tabacum* crude extracts and as aphrodisiac concoction ingredients in folklore may be justified but should be discouraged as its administration at high doses was observed to pose toxicological effects that may result in sexual dysfunction and even cause hazardous effects to users. Furthermore, molecular and clinical studies, as well as chemical identification and isolation of the bioactive compounds of these psychoactive plants, are recommended to evaluate their pharmacokinetic, pharmacodynamic, and toxicological mechanism of action to mediate the observed sexual function or dysfunction.

Funding None.

Declarations

Ethics approval The care and use of Laboratory Animals were approved by the Federal University of Technology Akure ethical committee, which was followed strictly and in compliance with the National Institute of Health guidelines. Ethical approval was obtained from the Centre for Research and Development (CERAD), Federal University of Technology, Akure with the number FUTA/ETH/2020/016.

Informed consent All the authors contributed substantially to the work, participated in the writing, and have seen and approved the submitted version.

Conflict of interest The authors declare no conflict of interest.

References

Abdurahman H, Adejumo O, Abdulmalik J (2019) Prevalence of psychoactive substance use among male adolescents in Southwest Nigeria. J Subst Abus 24(5):475–480. https://doi.org/10.1080/14659891.2019.1604840

Adefegha SA, Oboh G, Olopade EO (2021) β-caryophyllene improves sexual performance via modulation of crucial enzymes relevant to erectile dysfunction in rats. Toxicol Res 37(2):249–260. https://doi.org/10.1007/s43188-020-00061-2

Adefegha SA, Oboh G, Fakunle B, Oyeleye SI, Olasehinde TA (2018) Quercetin, rutin, and their combinations modulate penile phosphodiesterase-5′, arginase, acetylcholinesterase, and angiotensin-I-converting enzyme activities: a comparative study. Comp Clin Pathol 27(3):773–780. https://doi.org/10.1007/s00580-018-2664-y

Ademiluyi AO, Ogunsuyi OB, Oboh G, Agbebi OJ (2016) Jimson weed (*Datura stramonium* L.) alkaloid extracts modulate cholinesterase and monoamine oxidase activities *in vitro*: possible modulatory effect on neuronal function. Comp Clin Pathol 25(4):733–741. https://doi.org/10.1007/s00580-016-2257-6

Ademiluyi AO, Oyeniran OH, Jimoh TO, Oboh G, Boligon AA (2019) Fluted pumpkin (*Telfairia occidentalis*) seed modulates some markers of erectile function in isolated rat's corpus cavernosum: influence of polyphenol and amino acid constituents. J Food Biochem 43(11):1–11

Ademosun AO, Adebayo AA, Oboh G (2019) *Anogeissus leiocarpus* attenuates paroxetine-induced erectile dysfunction in male rats via enhanced sexual behavior, nitric oxide, and antioxidant status. Biomed Pharmacother 111:1029–1035

Akintunde JK, Oboh G, Akindahunsi AA (2015) Inhibition of key markers linked with spermatogenesis and cellular ATP by sub chronic exposure to leachate in a rat model. Arch Environ Contam Toxicol 68(1):159–168. https://doi.org/10.1007/s00244-014-0068-9

Andersson KE (2011) Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. Pharmacol Rev 63:811–859

Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M, Mastinu A (2018) *Cannabis sativa*: a comprehensive ethnopharmacological review of a medicinal plant with a long history. J Ethnopharmacol 227:300–315

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Caldwell RW, Rodriguez PC, Toque HA, Narayanan SP, Caldwell RB (2018) Arginase: a multifaceted enzyme important in health and disease. Physiol Rev 98(2):641–665

Castela Â, Costa C (2016) Molecular mechanisms associated with diabetic endothelial erectile dysfunction. Nat Rev Urol 13(5):266–274

Chen L, Shi G, Huang D, Li Y, Ma C, Shi M, Su B, Shi G (2019) Male sexual dysfunction: a review of literature on its pathological mechanisms, potential risk factors, and herbal drug intervention. Biomed Pharmacother 112:108585. https://doi.org/10.1016/j.biopha.2019.01.046

Cushman DW, Cheung HS (1971) Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 20(7):1637–1648

Dumbili EW (2020) Drug-related harms among young adults in Nigeria: implications for intervention. J Hum Behav Soc Environ 30(8):1013–1029. https://doi.org/10.1080/10911359.2020.1790462

Fasakin OW, Oboh G, Ademosun AO (2021) Neuromodulatory evaluation of commonly abused plants ex vivo: a comparative study. Comp Clin Pathol 30:671–680. https://doi.org/10.1007/s00580-021-03259-4

Fraga-Silva RA, Montecucco F, Mach F, Santos RAS, Stergiopulos N (2013) Pathophysiological role of the renin-angiotensin system on erectile dysfunction. Eur J Clin Invest 43(9):978–985

German CL, Fleckenstein AE, Hanson GR (2014) Bath salts and synthetic cathinones: an emerging designer drug phenomenon. Life Sci 97:2–8

Guohua H, Yanhua L, Rengang M, Dongzhi W, Zhengzhi M, Hua Z (2009) Aphrodisiac properties of Allium tuberosum seeds extract. J Ethnopharmacol 122:579–582

Hatzimouratidis K, Salonia A, Adaikan G, Buvat, J, Carrier S, El-Meliegy A, McCullough A, Torres LO, Khera M (2016) Pharmacotherapy for erectile dysfunction: recommendations from the Fourth International Consultation for Sexual Medicine (ICSM 2015) J Sex Med 13(4):465–488

Hayashi I, Morishita Y, Imai K, Nakamura M, Nakachi K, Hayashi T (2007) High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat Res Genet Toxicol Environ Mutagen 631(1):55–61

Heidelbaugh JJ (2010) Management of erectile dysfunction. Am Fam Physician 81(3):305–312

Jiménez DL, Chozas JML, Montoro AE, Corral MÁR, Guerra LC, González JPM (2019) Could renin-angiotensin-aldosterone system activation explain the amputations associated with canagliflozin? The nitric oxide hypothesis. Diabetes Res Clin Pract 148:10–13

Johnson LE, Balyan L, Magdalany A, Saeed F, Salinas R, Wallace S, Veltri CA, Swogger MT, Walsh Z, Grundmann O (2020) The

potential for kratom as an antidepressant and antipsychotic. Yale J Biol Med 93:283–289
Kelly SJ, Butler LG (1977) Enzymic hydrolysis of phosphonateesters. Reaction mechanism of intestinal 50-nucleotide phosphodiesterase. Biochemistry 16:1102–1104
Kenneth LB (2001) Principles and practice of endocrinology and metabolism, 3rd edn. Williams & Wilkins, Lippincott, Philadelphia, p 957
Klaassen C, Watkins J (2003) Casarett and Doull's essentials of toxicology. McGraw-Hill, New York, p 512
Lorenzen JM, Ückert S, Scheller F, Haller H, Kuczyk MA (2009) Effects of arginase inhibitors on the contractile and relaxant responses of isolated human penile erectile tissue. World J Urol 27:805–810
Lugnier C (2006) Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. Pharmacol Ther 109:366–398
Malviya N, Malviya S, Jain S, Vyas S (2016) A review of the potential of medicinal plants in the management and treatment of male sexual dysfunction. Andrologia 48:880–893. https://doi.org/10.1111/and.12677
Masuku NP, Unuofin JO, Lebelo SL (2020) Promising role of medicinal plants in the regulation and management of male erectile dysfunction. Biomed Pharmacother 130:110555. https://doi.org/10.1016/j.biopha.2020.110555
Miranda KM, Espay MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide: Biol Chem 5:62–71
Ojo OA, Ojo AB, Oyinloye BE, Ajiboye BO, Anifowose OO, Akawa A, Olaiya OE, Olasehinde OR, Kappo AP (2019) *Ocimum gratissimum* Linn. Leaves reduce the key enzymes activities relevant to erectile dysfunction in isolated penile and testicular tissues of rats. BMC Complement Med Ther 19(71):1–10. https://doi.org/10.1186/s12906-019-2481-0
Oboh G, Ademiluyi AO, Oyeleye SI, Olasehinde TA, Boligon AA (2017) Modulation of some markers of erectile dysfunction and malonaldehyde levels in isolated rat penile tissue with unripe and ripe plantain peels: Identification of the constituents of the plants using HPLC. Pharm Biol 51(1):1920–1926
Odubanjo VO, Olasehinde TA, Oyeleye SI, Oboh G, Boligon AA (2018) Seed extracts from *Myristica fragrans* (Nutmeg) and *Moringa oleifera* (Drumstick tree) inhibits enzymes relevant to erectile dysfunction and metal-induced oxidative damage in rats' penile tissues. J Food Biochem 42(1):1–9. https://doi.org/10.1111/jfbc.12452
Outhoff K (2009) Antidepressant-induced sexual dysfunction. South Afr Fam Pract 51:298–302
Passaglia P, Ceron CS, Mecawi AS, Antunes-Rodrigues J, Coelho EB, Tirapelli CR (2015) Angiotensin type 1 receptor mediates chronic ethanol consumption-induced hypertension and vascular oxidative stress. Vascul Pharmacol 74:49–59
Patrick-Iwuanyanwu KC, Onyeike EN, Wegwu MO (2010) Hepatoprotective effects of methanolic extract and fractions of African mistletoe Tapinanthus bangwensis (*engl. k. krause*) from Nigeria. EXCLI J 9:187–194
Perry NS, Houghton PJ, Theobald A, Jenner P, Perry EK (2000) In-vitro inhibition of human erythrocyte acetylcholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. J Pharm Pharmacol 52(7):895–902
Rincón J, Correia D, Arcaya JL, Finol E, Fernández A, Pérez M, Yaguas K, Talavera E, Chávez M, Summer R, Romero F (2015) Role of Angiotensin II type 1 receptor on renal NAD (P) H oxidase, oxidative stress and inflammation in nitric oxide inhibition induced-hypertension. Life Sci 124:81–90
Sasatomi K, Hiragata S, Miyazato M, Chancellor BM, Morris S, Yoshimura N (2008) Nitric oxide mediated suppression of detrusor overactivity by arginase inhibitor in rats with chronic spinal cord injury. Urology 72:696–700
Sommer F, Schulze W (2005) Treating erectile dysfunction by endothelial rehabilitation with phosphodiesterase 5 inhibitors. World J Urol 23:385–392
Tabit CE, Chung WB, Hamburg NM, Vita JA (2010) Endothelial dysfunction in diabetes mellitus: Molecular mechanisms and clinical implications. Rev Endocr Metab Disord 11(1):61–74
Weisshaar HD (1975) Normal ranges of alpha-HBDH, LDH, AP, and LAP as measured with substrate-optimated test charges. Med Welt 26:387–392
World Health Organization (2016) Progress report 2016: prevent HIV, test and treat all: WHO support for country impact (No. WHO/HIV/2016.24) Geneva: World Health Organization
Zhang C, Hein TW, Wang W, Chang CI, Kuo L (2001) Constitutive expression of arginase in microvascular endothelial cells counteracts nitric oxide mediated vasodilatory function. FASEB J 15:1264–1266
Zhao Y, Vanhoutte PM, Leung SWS (2015) Vascular nitric oxide: Beyond eNOS. J Pharmacol Sci 129(2):83–94