RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2025.063

ScienceAsia 51 (4): 2025: ID 2025063: **1–10**

Aphrodisiac potential of *Derris scandens* (Roxb.) Benth. stem extract on mating behaviors in immobilization-induced stress male rats

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Received 5 Oct 2023, Accepted 22 May 2025
Available online 13 Jul 2025

ABSTRACT: This study aimed to investigate the aphrodisiac potential effects of *Derris scandens* (Roxb.) Benth. stem extract (DE) on the mating behaviors in immobilization-induced stress male rats. Forty-eight male Wistar rats were divided into 6 groups: Normal control, Stressed control, Stressed rats that received DE 200, 400, and 800 mg/kg/day for 14 days, and Stressed rats that received sildenafil citrate 5 mg/kg/day. The rats were exposed to immobilization for 6 h per day for 14 days to induce stress. Mating behaviors were studied on Day 0 and Day 14. On Day 15, blood was collected by heart puncture for testosterone and cortisol level evaluation, and sperm was collected from both sides of the caudal epididymis and vas deferens. Neurotransmitter levels extracted from the brain were measured by HPLC-ECD analysis. Superoxide dismutase (SOD) was evaluated using SOD Assay Kit. The results showed that the administration of DE significantly motivated the mating behaviors by reducing the mount latency and intromission latency and increasing the intromission number. The increases in sperm count and SOD activity were only observed in the stressed rats treated with DE 400 mg/kg. The stressed rats that received 800 mg/kg of DE showed a significant increase in dopamine levels and a decrease in norepinephrine levels. Our studies provide experimental evidence that 400 and 800 mg/kg DE can be effective in improving male rat sexual performance.

KEYWORDS: aphrodisiac, *Derris scandens*, erectile dysfunction, mating behaviours, stress

INTRODUCTION

Erectile dysfunction (ED) is defined as 'an inability to achieve and sustain a penile erection sufficient for sexual performance' [1]. The Massachusetts Male Ageing Study (MMAS) between 1987 and 2004 reported an overall prevalence of 52% ED in men aged 40 to 70 years [2]. Stress has become one of the important factors causing ED [3]. It has been reported that exposure to stress can reduce plasma luteinizing hormone levels, testosterone secretion, reproductive function, spermatogenesis, and libido [4, 5]. The study on sexual behaviors of male rats submitted to chronic immobilization (IMB)-induced stress indicated that prolonged IMB increased the mount latency and the number of thrusts [6]. Moreover, long-term psychological stress reduces the catecholamine levels and their metabolites, including dopamine, homovanillic acid (HVA), 5,6-dihydroxyphenylacetic acid (DOPAC), and norepinephrine in the brain, especially in the medial preoptic area (MPOA). These lead to an impairment of male rat sexual behaviors [7]. In addition, it has been reported that stress causes an increase in reactive oxygen species (ROS) in the reproductive tract, leading to oxidative stress. The increase in ROS production induces DNA damage and alters antioxidant enzyme activity in the testes, which can lead to reduced sperm motility, viability, capacitation, and function, ultimately resulting in male infertility [8, 9]. The enzymatic antioxidants that scavenge ROS production in the testes include superoxide dismutase (SOD) enzymes, catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR). Many studies have suggested that antioxidant enzymes are necessary for the function and existence of spermatozoa [8–10]. Antioxidant enzymes have recently been identified as a promising target for ED therapy [11], and antioxidant agents have been shown to increase sperm motility and reduce oxidative stress-induced sperm damage [12].

The treatment of ED has currently focused on the mechanisms involved in the relaxation of the corpus cavernosum smooth muscle. The biochemical and physiological functions of phosphodiesterase 5 (PDE5) enzymes, related to the relaxation mechanism, could enhance erectile function sufficiently for normal sexual activity. The PDE5 inhibitor drug has been used as a first-line treatment for ED and so is a target of interest for the treatment of ED [13]. It was also found that

PDE5 inhibitors can reduce oxidative stress by decreasing superoxide formation and increasing cGMP, cAMP, and glutathione levels in rabbit corpus cavernosum and hypertensive rats [11]. In addition, PDE5 has also been found to increase SOD and CAT activity [14, 15]. However, some unpleasant side effects of the PDE5 inhibitor drug have been reported, including headache, flushing, stuffy nose, nasopharyngitis, and dyspepsia. Also, rare but serious adverse effects have been reported, including priapism, visual abnormality, and hearing loss [16–18]. Thus, the search for new PDE5 inhibitors, especially from medicinal plants, is worth considering. Of specific interest to our research, the plant *Derris scandens* (Roxb.) Benth. may be a source of new PDE5 inhibitors.

Derris scandens (Roxb.) Benth. is known in Thailand as thao-wan-priang, and its common names include jewel vine, hog creeper, and Malay jewel vine. It is a woody vine belonging to the genus *Derris* of the Fabaceae family. Its stem has long been used in Thai traditional medicine for the treatment of various ailments such as musculoskeletal pain and antitussive, as well as an expectorant and a nourishing agent [19]. The traditional use of *D. scandens* is not only for relieving muscle pain but also for nourishing the fire elements in our bodies. In Thai traditional medicine, the fire element plays a role in the motivation of the sex drive; therefore, the increase of the fire elements by *D. scandens* evokes a sexual desire. Our previous study reported that the ethanol extract and isoflavone isolated from the *D. scandens* stem showed PDE5 inhibitory activity. The IC_{50} value of the *D. scandens* stem extract against PDE5 was 7 µg/ml. The PDE5 inhibitory activity of the isoflavone-isolated compound osajin was high, with an IC_{50} value of approximately 4 µM. For 4′,5,7-trihydroxybiprenylisoflavone, the IC_{50} was around 8 µM, while derrisisoflavone A exhibited an IC_{50} value of approximately 9 µM. Lupalbigenin showed weaker PDE5 inhibition with an IC_{50} value of approximately 16 µM. Derrisisoflavone A and lupalbigenin are the major components of the ethanol extract of *D. scandens* stem. A previous study suggested that *D. scandens* may represent a promising source for PDE5 inhibitor drug development for ED therapy [20]. This study agrees with the study by Bhandari et al [21] which reported the potential fraction from *D. scandens* containing 25 active compounds on PDE5A1 inhibitory activity. However, no study has yet been published to demonstrate the effect of *D. scandens* stems on sexual motivation. For this reason, the objectives of our research were to investigate the ability of *D. scandens* to improve male rats' sexual dysfunction induced by stress. Various factors, which play important roles in rats' mating behaviors, were evaluated to explore the potential mechanism of *D. scandens* stem on the mating behaviors in immobilization-induced stress male rats, including their sexual behavior parameters, serum testosterone, sperm count and sperm motility, and catecholamine levels. The antioxidant enzyme SOD in the testes was investigated for its potential benefits for preventing ED in stressed rats.

MATERIALS AND METHODS

Chemicals and reagents

Estrogen (β-estradiol 3-benzoate) and progesterone (4-pregnene-3,20-dione), both of certified reference material and pharmaceutical secondary standard quality, were procured from Sigma-Aldrich (St. Louis, USA). Sodium pentobarbital (Nembutal) was purchased from CEVA (Shanghai, China). Isolated compounds from *D. scandens* stem (derrisisoflavone A and lupalbigenin, purity > 95%) were provided as gifts from Prof. Dr. Apichart Suksamrarn of the Faculty of Sciences, Ramkhamhaeng University, Thailand.

Plant material

Fresh stems of *Derris scandens* (Roxb.) Benth., called thao-wan-priang in Thailand, were collected from Phitsanulok Province, Thailand, in the period between April to October 2016. Botanical identifications were performed by Dr. Pranee Nangngam from the Department of Biology, Faculty of Science, Naresuan University (NU), Thailand. The voucher specimen (specimen No. 004331) was deposited at PNU Herbarium, Faculty of Science, NU, Thailand.

Preparation of the extract

The dried and powdered *D. scandens* (1 kg) was macerated with 95% ethanol (4 l) for 24 h at room temperature, with agitation at 300–350 rpm, followed by filtration through filter paper. The residue obtained after filtration was subjected to a second extraction. Subsequently, the combined extracts were concentrated by removing the organic solvent under reduced pressure at a controlled temperature of 30–35 °C, yielding an ethanolic extract of *D. scandens* (DE) with a recovery rate of 2.8%.

Determination of active compounds in the extract using LC-QTOF-MS

The identification of the major active compounds present in the extract, derrisisoflavone A and lupalbigenin, was conducted using LC-QTOF-MS analysis, as previously described [20]. The amounts of derrisisoflavone A and lupalbigenin in the DE were $3.03 \pm 1.01\%$ w/w and $3.49 \pm 1.12\%$ w/w, respectively.

Animals

Male and female Wistar rats (7–9 weeks old), specific pathogen-free (SPF) grade, were supplied by M-CLEA Bioresource Co., Ltd., located in Bang Phli, Samut Prakarn Province. The animals were housed in groups of 3–4 per cage at the NU Center for Animal Research (NUCAR), which holds full accreditation from AAALAC

International. The animal room was maintained at a constant temperature of 22 ± 1 °C, with relative humidity at 55 ± 10%, under a 12-h reverse dark-light cycle (light on: 09:00 PM–09:00 AM; light off: 09:00 AM–09:00 PM) to facilitate behavior testing. The rats were provided with a standard laboratory pellet diet (CP082) and had ad libitum access to reverse osmosis (RO) water.

Experiments involving the rats were conducted in accordance with the Ethical Principles for the Use of Animals for Scientific Purposes, issued by the National Research Council of Thailand (NRCT). The study protocol received approval from the NU Animal Care and Use Committee (approval number: 590712; approval date: October 31, 2016).

Preparation of female rats for studying their mating behaviors

To prevent pregnancy, 14 female rats were subjected to bilateral ovariectomy at least 2 weeks before conducting mating behaviors. All the female rats were made receptive through the administration of estradiol benzoate (0.1 ml of 25 µg) via subcutaneous injection 48 h before pairing, followed by progesterone (0.1 ml of 500 µg) administered subcutaneously 4 h before pairing.

Stress introduction procedure

The method was modified from Almeida et al [22]. Briefly, the male rats were stressed by immobilizing the animal inside a restraint-plastic tube, 15.5 cm in length and 4.5 cm in diameter, for 6 h a day during the light-off period starting at 8:00 am each day for 14 consecutive days.

Animal grouping and extract administration

The male rats were randomly divided into 6 groups of 8 each and orally administered as follows. Group I animals served as normal controls and received only the vehicle, i.e., propylene glycol (PG). Group II rats served as the stress control and received IMB stress plus PG. Group III rats received IMB stress and DE at the dose of 200 mg/kg/day, while Group IV and Group V received 400 and 800 mg/kg/day, respectively. Group VI animals, serving as positive control, were subjected to IMB stress and administered sildenafil citrate at the dose of 5 mg/kg, 30 min prior to the mating behavior test.

DE treatments and stress exposure were administered once daily over a 14-day period (Day 1–Day 14). Following stress exposure, the animals were released from their restraint-plastic tubes and provided with a 30-min recovery period. Subsequently, the test substances were administered via oral gavage, and the animals were returned to their cages prior to the mating behavior test.

Mating behavior evaluation

Their mating behaviors were assessed on Day 1, approximately 30 min after substance administration, and again on Day 14, using the same procedure. Each male rat was individually placed in an observation cage (a clear glass box measuring 70 × 35 × 35 cm) and allowed to acclimate to the environment for approximately 5 min. An estrous female rat was then introduced into each observation cage, and mating behaviors were continuously recorded using a digital video camera for a duration of 30 min. The mating behaviors of the rats were independently evaluated by 2 investigators who were as unconnected as practicable to ensure unbiased observations. The results from these evaluations were then pooled and analyzed by a third investigator. The assessed mating parameters include the following parameters:

(1) Mount latency (ML): The time from the introduction of the female to the first mount by the male.
(2) Mounting number (MN): The number of mounts without intromission from the time of the introduction of the female until ejaculation.
(3) Intromission latency (IL): The time from the introduction of the female to the first intromission by the male.
(4) Intromission number (IN): The number of intromissions from the time of female introduction to ejaculation.
(5) Ejaculation latency (EL): The time from the first intromission to ejaculation.
(6) Ejaculation number (EN): The number of ejaculations characterized by longer, deeper pelvic thrusting and slow dismount, followed by a period of inactivity.
(7) Postejaculatory interval (PEI): The time from ejaculation to the next intromission.

Measurement of body weight and sexual organ weight

The body weight (BW) of each male rat was recorded on Days 0, 7, and 14. The percentage of BW loss was calculated using the formula: 100 × ((average of BW on Day 0 − average of BW on Day 14)/Average of BW on Day 0), based on 8 rats per group. Following blood collection at termination, the sexual organs—including the penis, testes, epididymis, and vas deferens—were carefully excised and weighed. The organ weights (in grams) were normalized to 100 g of BW and expressed as a percentage of organ weight relative to BW.

Measurement of hormones in the blood

All male rats were sacrificed on Day 15, within 24 h after the last measurements on Day 14. The rats were anesthetized by intraperitoneal (i.p.) injection of Nembutal (100 mg/kg BW), then blood was taken from the heart by cardiac puncture, which euthanized them, and the blood was collected into heparinized tubes

and stored at 4 °C before sending to Biolab Medical Technic Clinic, Phitsanulok, Thailand, for testosterone and cortisol measurement.

Measurement of semen parameters

The spermatozoa samples were collected from both sides of the caudal epididymis and vas deferens. The collected samples were suspended in 1 ml of 1X phosphate-buffered saline (PBS) at 37 °C for 30 min. Ten microliters of the sample solutions were then transferred to the hemocytometer (Counting Chamber, Makler, USA) and analyzed under a light microscope connected to a computer. The sperm analyses were done to evaluate sperm numbers and sperm motility. The sperm numbers were randomly counted from 3 rows, and the calculation was performed according to the number of counted cells and hemocytometer dimensions. The results are expressed as the mean count in millions of sperm per milliliter. For motility evaluations, the motile and immotile sperm were classified. The results were then calculated and expressed as the percentage of motile sperm in the total sperm count.

Measurement of superoxide dismutase (SOD) activity in testes

The testes were weighed, rinsed with 1 M PBS (pH 7.4), and homogenized (Ultra-Turrax T8, Ika-Werke GmbH & Co., Ltd., Staufen im Breisgau, Germany) in 10 ml of cold 2 mM HEPES buffer (pH 7.2) per gram of tissue. The buffer contained 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose. The homogenate was subjected to centrifugation (Universal 320R, Hettich, Germany) at $1500 \times g$ for 5 min. The resulting supernatant was further centrifuged at $10000 \times g$ for 15 min at 4 °C. Total SOD activity in the testicular homogenates was quantified using an SOD Assay Kit (Cayman Chemical, MI, USA) following the manufacturer's protocol. Absorbance was measured at 440 nm with a microplate reader (EON, Biotek, USA).

Measurement of neurotransmitter levels in rat brain using HPLC-ECD

Upon termination, the brain tissue was excised and rinsed in cold (4 °C) normal saline. It was then weighed and stored at −80 °C to prevent enzymatic degradation before sample preparation [23]. The whole brain tissue of each rat was weighed and homogenized (Ultra-turex T8, Ika-werke GMBH & Co., Ltd.) in 0.1 M of perchloric acid (0.1 g/ml) and centrifuged (Labofuge 400R, Heraeus Instruments, Germany) at 14,000 rpm for 20 min at 4 °C. The supernatant was then used as a source for measuring norepinephrine (NE), dopamine (DA), and serotonin (5-HT) levels [23].

The HPLC-ECD analysis was performed using a Shimadzu CBM-20A HPLC system (Kyoto, Japan) equipped with Shimadzu LC-20AD pumps, DGU-20A degassing unit, SIL-20AC prominence HPLC auto-samplers, CTO-10ASVP column oven, and coupled to an ESA Coulochem III electrochemical detector. The temperature of the auto-sampler was maintained at 4 °C. The optimal electrical potential settings were: E1 −175 mV and E2 +220 mV. Chromatographic separations were achieved on an ACE C18-AR HPLC column (4.6 mm × 250 mm). The mobile phase consisted of 3% methanol in water, 3% acetonitrile in water, 0.15 mM ammonium acetate, and 0.1 mM EDTA, buffered to a pH of 6 with 1 M acetic acid. The flow rate was set at 1 ml/min. A post-time of 3 min was allowed for system re-equilibration before the next injection. The injection volume was 20 µl. The stock solution of each standard compound was freshly prepared by dissolving in 0.1 M perchloric acid to obtain a concentration of 10 mg/ml. These standard solutions were further mixed and diluted with water to make standard calibration curves which ranged from 16–1,024 ng/ml. Peaks in the sample solution were identified by comparing the retention time of each peak in the standard solution of NE, DA, and 5-HT.

Statistical analysis

All values were expressed as the mean ± standard error of measurement (SEM). Statistical analysis was conducted using Student's unpaired t-test and one-way analysis of variance (ANOVA), followed by Duncan's new multiple range test to detect inter-group differences. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of DE on the mating behaviors of stress-induced ED rats

Mating behaviors were studied on Day 1 and Day 14 after immobilization. As shown in Fig. 1, ML and IL on Day 1 and Day 14 of the stress control group were significantly longer than those of the normal control group ($p < 0.05$). The IN of the stress control group was significantly lower than that of the normal control group. These indicated the ED suffering of the rats in the stress control group [6]. The reduction in ML and IL of the stressed rats that had been treated with DE 200, 400, or 800 mg/kg on Day 1 and Day 14 was compared to the reduction in ML and IL of the stressed rats treated with the vehicle. This comparison suggested an increase in the sexual activity of the treated male rats and the aphrodisiac properties of DE [24]. Additionally, these mating behaviors are consistent with the results observed in stressed rats treated with sildenafil. This similarity may be attributed to the PDE5 inhibitory effect of the extract, which leads to increased blood flow to the male reproductive organs [25]. However, the treatment with DE did not exhibit a dose-dependent manner, which may be attributed to the extract containing multiple active compounds that interact in complex ways, potentially leading to non-

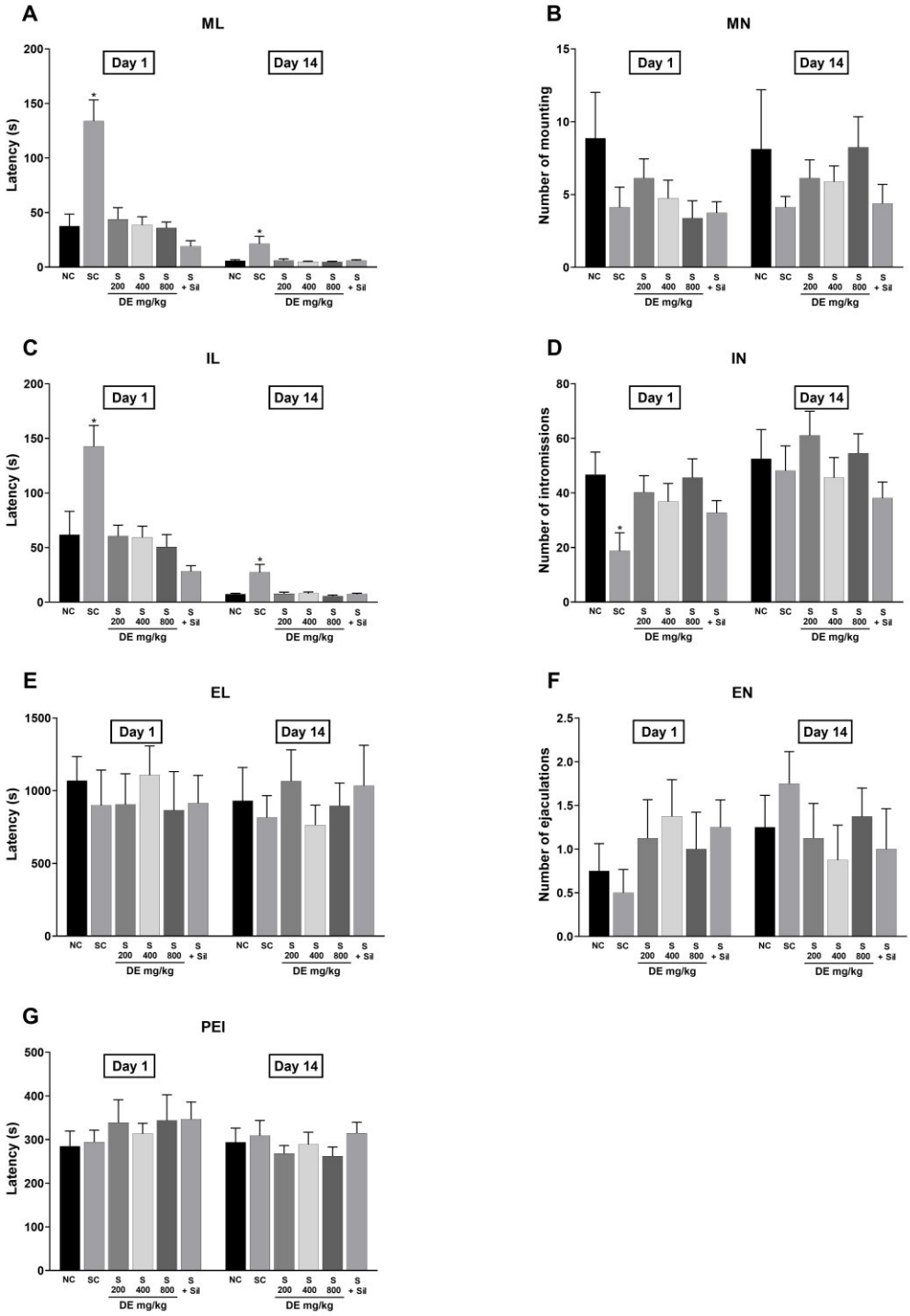

Fig. 1 Mating behavior parameters in male rats experiencing immobilization-induced stress after a single dose (Day 1) and after 14 days (Day 14) of treatment with 200, 400, or 800 mg/kg *D. scandens* extract (DE), sildenafil 5 mg/kg (Sil), or vehicle (SC). Latencies are expressed in seconds (s), and frequencies are expressed as the number of events; (A) ML = Mount latency, (B) MN = Mount number, (C) IL = Intromission latency, (D) IN = Intromission number, (E) EL = Ejaculation latency, (F) EN = Ejaculation number, and (G) PEI = Postejaculatory interval. Data represent mean ± SEM (number of rats = 8 per group). One-way ANOVA followed by Duncan's new multiple range test, * $p < 0.05$ vs. NC.

Table 1 Effect of stress on BW (g) before starting the experiments (Day 0), after 7 days (Day 7), and after 14 days (Day 14) of treatment, and percentage of weight loss of stress-induced ED rats after 14 days of treatment.

Group	Day 0	Day 7	Day 14	% Weight loss
Group I (Normal control)	451.4 ± 24.51	455.6 ± 27.27	441.9 ± 22.74	2.10 ± 1.44
Group II (Stress + vehicle)	498.9 ± 39.52	471.8 ± 42.79	457.9 ± 46.10	8.22 ± 1.71*
Group III (Stress + DE 200)	510.3 ± 7.63	468.3 ± 22.07	464.2 ± 22.83	9.03 ± 1.26*
Group IV (Stress + DE 400)	508.7 ± 25.47	485.2 ± 24.58	475.5 ± 25.28	6.52 ± 1.07*
Group V (Stress + DE 800)	503.6 ± 16.69	473.8 ± 12.66	460.3 ± 20.29	8.61 ± 1.42*
Group VI (Stress + sildenafil)	482.1 ± 41.85	459.9 ± 40.27	459.2 ± 48.23	4.95 ± 1.00

Values are expressed as the mean ± SEM, 8 rats/group. * p-value < 0.05 compared to the normal control group.

Table 2 Effect of *D. scandens* extract (DE) on the percentage of organ weight per BW of stress-induced ED rats after 14 days of treatment.

Group	Penis	Testis	Epididymis	Vas deferens
Group I (Normal control)	0.10 ± 0.00	0.81 ± 0.02	0.13 ± 0.00	0.07 ± 0.01
Group II (Stress + vehicle)	0.09 ± 0.00	0.81 ± 0.03	0.12 ± 0.01	0.07 ± 0.01
Group III (Stress + DE 200)	0.09 ± 0.01	0.77 ± 0.01	0.12 ± 0.01	0.08 ± 0.01
Group IV (Stress + DE 400)	0.08 ± 0.00	0.76 ± 0.01	0.11 ± 0.01	0.08 ± 0.00
Group V (Stress + DE 800)	0.10 ± 0.00	0.80 ± 0.01	0.12 ± 0.00	0.08 ± 0.01
Group VI (Stress + sildenafil)	0.08 ± 0.00	0.78 ± 0.02	0.12 ± 0.00	0.07 ± 0.00

Values are expressed as the mean ± SEM, 8 rats/group.

linear dose-response relationships. Furthermore, at higher doses, the receptors targeted by the extract may become saturated, resulting in no further increase in effect [26].

Any medicinal plant with aphrodisiac tendencies should produce statistically significant increases in the indices of sexual vigor of MN and IN and significant decreases in ML and IL. These are indicators of stimulation of sexual arousal, motivation, and vigor. The significant decrease in ML and IL and the significant increase in the computed male sexual behavior parameters of the percentage of mountings, percentage of intromissions, percentage of rats that ejaculated, and the reduction in intercopulatory efficiency can indicate a sustained increase in sexual activity and aphrodisiac property inherent in the plant extract. On Day 1, the IN of the stressed rats treated with DE 200 and 800 mg/kg was significantly higher than that of the stress control group, while the dose of 400 mg/kg tended to increase IN, but this was not significantly different from the stress control group. This indicated that DE affects the stimulation of sexual motivation, sexual arousal, and vigor [24]. However, a significant increase in IN of the stressed rats treated with DE on Day 14 was not observed.

EL, EN, and PEI values were not significantly different among the groups. Consistent findings have been reported in previous studies investigating the effects of certain plant extracts on stress-induced ED caused by IMB [27, 28]. Treating stressed rats with 200 mg/kg of *Cynodon dactylon* methanol extract for 30 days could improve sexual function by increasing MN and decreasing IL without an effect on ML, IN, EL, and EN [27]. Seven-day oral administration of 10 mg/kg *Moringa oleifera* leaf extract could increase sexual activities by decreasing IL and increasing IN without affecting ML, MN, EL, and EN [28]. These studies suggest that plant extracts can increase the sexual functions of stressed rats through various mechanisms. Certain plant extracts may promote arousal by increasing MN, reducing ML, or decreasing IL, while others may improve penile erection efficiency by increasing IN. The present study demonstrated the sexual motivation effects, sexual activity effects, and aphrodisiac properties of DE in stressed rats by reducing ML and IL. Furthermore, DE increased sexual motivation, arousal, and vigor by increasing IN [24].

Effect of stress on body weight loss and sexual organ weight

The impact of stress on BW and sexual organ weight was evaluated following treatment with IMB for 6 h daily over a 14-day period. A significant reduction in BW was observed in all IMB-induced stressed rats compared to the normal control group, except for the rats treated with sildenafil. The relative weights of the penis, testes, epididymis, and vas deferens across all groups did not differ significantly from those of the normal control group, indicating that stress did not affect the sexual organs of the rats. The results are presented in Table 1 and Table 2.

Effect on sperm

Sperm was collected from both sides of the caudal epididymis and vas deferens. The spermatozoa concentration in the cauda epididymis generally declines

Table 3 Effect of DE on the mean count of sperm and percentage of sperm motility of stress-induced ED rats after 14 days of treatment.

Group	Mean count ($\times 10^6$ sperm/ml)	% Sperm motility
Group I (Normal control)	125.00 ± 15.84	40.42 ± 1.18
Group II (Stress + vehicle)	56.50 ± 8.28*	36.17 ± 2.12
Group III (Stress + DE 200)	92.33 ± 7.69	29.59 ± 3.76*
Group IV (Stress + DE 400)	109.04 ± 20.89#	38.28 ± 2.17
Group V (Stress + DE 800)	88.58 ± 12.11	27.57 ± 2.16*,#
Group VI (Stress + sildenafil)	88.67 ± 11.91	35.72 ± 2.39

Values are expressed as the mean ± SEM, 8 rats/group. * p-value < 0.05 compared to the normal control group. # p-value < 0.05 compared to the stress control group.

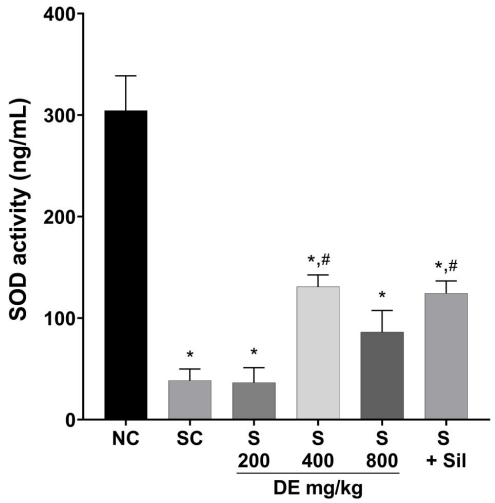

Fig. 2 Mean total SOD activity in the testes of stress-induced ED rats. Stress exposure (SC: stress + vehicle) significantly decreased SOD activity compared to the Normal control (NC). Stressed rats treated with 400 mg/kg *D. scandens* extract (DE) and 5 mg/kg sildenafil (Sil) showed significantly increased SOD activity compared to SC. All values are presented as the mean ± SEM (number of rats = 6 per group). * $p < 0.05$ vs. NC and # $p < 0.05$ vs. SC.

following IMB stress [6]. Our findings revealed that the mean sperm count in the stress control group was significantly lower than that observed in the normal control group (Table 3).

Interestingly, administration of 400 mg/kg DE mitigated the reduction in sperm counts observed in stress-induced rats, although it did not influence sperm motility. Notably, stress did not affect sperm motility in this experiment. However, rats treated with a higher dose of DE (800 mg/kg) exhibited a significantly lower percentage of sperm motility compared to other groups. These findings highlight the potential toxicity of DE at higher doses and warrant caution. Nevertheless, a previous study reported that DE at an even higher dose (1,000 mg/kg) demonstrated antinociceptive properties in rats without causing adverse effects [29]. Previous studies have explored the potential of plant extracts to increase sperm count in stressed rats. Notably, methanol extract of *C. dactylon* at a dose of 200 mg/kg was found to enhance sperm count in stressed rats, whereas treatment with a lower dose (100 mg/kg) or with other solvent extracts, such as aqueous and benzene extracts, did not yield similar results [27]. The increase of spermatozoa was also observed in the stressed rats treated with *M. oleifera* leaf extract at a high dose (250 mg/kg) but not with the lower doses (10 and 50 mg/kg) [28].

SOD activity in testes of stress-induced ED rats

Stress has been demonstrated to induce oxidative stress, resulting in the diminished activity of antioxidant enzymes, including SOD [8]. PDE5 inhibitors have been shown to mitigate oxidative stress and enhance the activity of antioxidant enzymes such as SOD and CAT [11, 15]. In the present study, as illustrated in Fig. 2, the mean total SOD activity in the testes of stressed rats treated with all doses of DE, as well as the vehicle and sildenafil, was significantly lower than that of the normal control group ($p < 0.05$). Notably, stressed rats treated with 400 mg/kg DE and the positive control, sildenafil, exhibited a significantly higher mean total SOD activity compared to the stressed control group. Conversely, administration of a high dose of DE (800 mg/kg) showed a tendency to increase SOD activity, but the difference was not statistically significant relative to the stressed control group. Moreover, DE at a dose of 200 mg/kg did not influence SOD activity.

Effect of DE on hormones in the blood of stress-induced ED rats

The effect of DE on cortisol and testosterone levels of the stress-induced ED rats is shown in Table 4. The cortisol hormone level of the stress control rats was significantly higher than that of the normal control group. The effect of DE at the doses of 200, 400, and 800 mg/kg and sildenafil at the dose of 5 mg/kg, to attenuate the increase of cortisol induced by stress, was observed. There have been reports that the increase

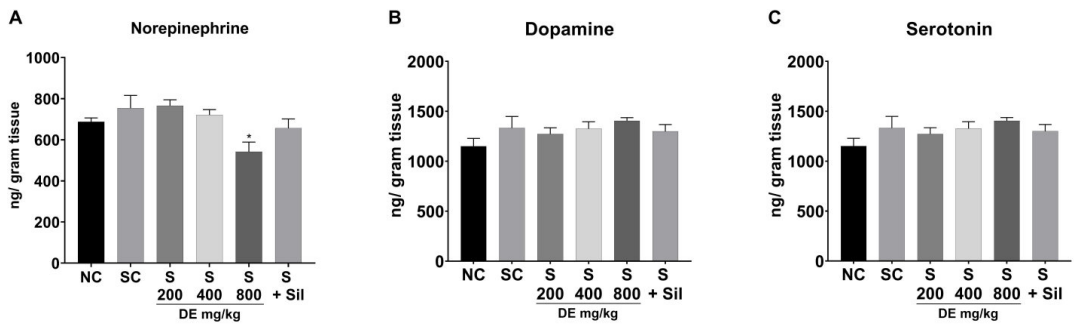

Fig. 3 Effect of *D. scandens* extract (DE) on neurotransmitter levels of stress-induced ED rats after 14 days of treatment with 200, 400, or 800 mg/kg *D. scandens* extract (DE), sildenafil 5 mg/kg (Sil), or vehicle (SC). All values are presented as the mean ± SEM (number of rats = 8 per group). * $p < 0.05$ vs. NC.

Table 4 Effect of DE on cortisol and testosterone levels in the blood of stress-induced ED rats after 14 days of treatment.

Group	Cortisol (ng/ml)	Testosterone (ng/ml)
Group I (Normal control)	9.1 ± 0.8	3.5 ± 0.8
Group II (Stress + vehicle)	11.8 ± 1.0*	2.9 ± 0.7
Group III (Stress + DE 200)	8.4 ± 0.5#	4.0 ± 0.6
Group IV (Stress + DE 400)	7.8 ± 0.6#	3.1 ± 0.5
Group V (Stress + DE 800)	8.8 ± 1.0#	2.7 ± 0.4
Group VI (Stress + sildenafil)	8.0 ± 1.0#	2.2 ± 0.2

Values are expressed as the mean ± SEM, 8 rats/group.
* p-value < 0.05 compared to the normal control group.
# p-value < 0.05 compared to the stress control group.

in cortisol levels induced by stress can directly inhibit testosterone production [30, 31]. Even though it has been reported that sildenafil increases the levels of serum testosterone [32], sildenafil administration to stressed rats failed to increase testosterone levels [33]. In addition, the study of Yazawa et al [34] reported that the testosterone levels of rats subjected to IMB stress were increased during the IMB induction and then returned to a normal state after 24 h of IMB. In the present study, the blood sample for testosterone level evaluation was collected after 20 h of IMB. Our results show that the testosterone levels of the rats treated with stress decreased, but the total testosterone levels were not significantly different from those of the control group. All doses of DE and sildenafil did not affect the level of testosterone in the stress-induced ED rats, which may be due to the recovery of the testosterone level following the last IMB induction to the pre-IMB phase [34]. Therefore, the levels of testosterone during the IMB induction phase should be evaluated.

Effect of DE on catecholamine levels in the rat brain

The levels of NE, DA, and 5-HT in the whole brain were analyzed across all groups. Previous studies have reported that stress can elevate the release of NE [31, 35] while decreasing DA and 5-HT levels in animals. The PDE5 inhibitor drug was found to enhance dopaminergic and serotonergic activity in rat brains [36, 37]. Our findings revealed no significant changes in these neurotransmitters across all groups. The stress induced by IMB in this experiment did not notably alter the levels of the analyzed neurotransmitters (Fig. 3). However, a decrease in NE levels and a slight increase in DA levels were observed in the stressed rats treated with 800 mg/kg of DE. These observations suggest that DE may play a role in modulating sexual arousal through the central nervous system [38–40]. Nonetheless, further research is required to elucidate and confirm these effects.

CONCLUSION

The findings of this study demonstrated that oral administration of DE significantly enhanced mating behaviors by reducing mount latency and intromission latency in IMB-induced stress erectile dysfunction (ED) in rats. An improvement in the efficiency of penile erection and penile orientation was evidenced by an increase in intromission numbers. Additionally, treatment with 400 mg/kg of DE increased sperm count and the number of viable sperm in stressed rats. Enhanced SOD activity in the testes was also observed exclusively in stressed rats treated with 400 mg/kg of DE, while administration of 800 mg/kg of DE elevated dopamine levels. However, the effects of DE on testosterone levels and neurotransmitter activity remain unclear. Furthermore, DE extract exhibited a PDE5 inhibitory effect in an *in vitro* study, though the underlying mechanism regarding PDE5 levels in penile tissue was not investigated. This study is the first to suggest the potential of DE as an aphrodisiac

in male rats. Nonetheless, further research is required to evaluate the effects of DE on cGMP levels, PDE5 activity in penile tissue and testes, penile blood flow, and intracavernous pressure. Additionally, it is crucial to analyze specific brain regions and the metabolites of neurotransmitters such as epinephrine, dopamine, and serotonin. Investigating the pathways involved in the mechanism of action would also provide a more comprehensive understanding.

Acknowledgements: This work was supported by the Thailand Science Research and Innovation (TSRI) [grant numbers DBG608005, DBG5980001, and IRN58W0005], NU, and the Centre of Excellence for Innovation in Chemistry (PERCH-CIC). Many Thanks go to Mr. Roy I. Morien of the NU Graduate School and Mr. Peter Barton of the Division of International Affairs and Language Development of NU for their editing of the grammar, syntax, and general English expression in this paper.

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