academicJournals

Vol. 7(32), pp. 2370-2379, 25 August, 2013
DOI: 10.5897/JMPR12.821
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Molecular mechanisms that underlie the sexual stimulant actions of ginger (*Zingiber officinale* Rosocoe) and garden rocket (*Eruca sativa* L.)

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Accepted 23 July, 2013

The effects of extracts and subfractions of *Zingiber officinale* rhizomes, *Eruca sativa* seeds and sildenafil on the sexual behavior of male rats and their effects on the intracavernosal pressure (ICV), intracavernosal cyclic cyclic guanosine monophosphate (GMP) and dihydrotestosterone plasma level were examined. The sexual behavior was followed for four hours using infra-red video cameras to quantify the effects on various male sexual behaviors. The results revealed that the active subfraction in case of *Z. officinale* was the hexane fraction of the chloroformic extracts (C/H) whereas that of *E. sativa* seeds was the acetonitrile fraction of the alcoholic extracts (A/Ac). (C/H), (A/Ac) and sildenafil significantly increased the total sexual stimulation index from 53.8 ± 2.7 (control) to 116 ± 3.8, 253 ± 2.7 and 401 ± 30.1, respectively. They significantly increased the index of successful mounting and ejaculation from 2.6 ± 0.5 (control) to 19 ± 0.5, 13 ± 0.6 and 18 ± 1.7, respectively. They significantly increased the cyclic GMP level from 0.94 ± 0.07 (control) to 2.81 ± 0.19, 2.65 ± 0.14 and 3.66 ± 0.19 ng/mg wet weight tissue, respectively. Furthermore, (C/H) increased plasma dihydrotestosterone level. Other treatments did not affect this parameter. (C/H), (A/Ac) and sildenafil increased the (I.CV) pressure. Both extracts and sildenafil acted via an increase in cyclic GMP with an additional increase in dihydrotestosterone release in case of (C/H).

Key words: Ginger, garden rocket, *Eruca sativa*, *Zingiber officinale*, sexual behavior, intracavernosal pressure, cyclic GMP (cyclic guanosine monophosphate), dihydrotestosterone.

Introduction

Since ancient times, man sought the natural products round him to discover aphrodisiac products that stimulate libido, sex drive and sexual performance. He tried consumption of herbs, plants, fruits, vegetables, marine products (caviar, kelp, lobster, oyster, cucumber etc), reptiles (for example, snakes), bees' products (honey, propolis) and even inorganic minerals for example, zinc sulphate. In the registered history, one can read throughout the registered folk medicine the names of various plants that are claimed to be aphrodisiacs (Chorpa et al., 1956; Madan et al., 1966; Said et al., 1996). The list of such plants is long but what strikes one is the

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continuous repetition of certain herbs in all of the investigated culture. Some of these included ginger rhizomes from *Zingiber officinale*, saffron stigmas from (*Crocus sativus*), garden rock seeds from *Eruca sativa* and the aerial parts of the mangrove plant *Avicennia marina*. Survey of the published literature via the Science Finder did not reveal direct studies in animals or man that address the sexual stimulant effect of the above four plants with the exception of an incomplete study regarding saffron in rats (Hosseinzadeh et al., 2008) and another in human volunteers (Shamsa et al., 2009). This paper is designed to address the aphrodisiac actions and the mechanisms underlying two of them namely ginger and *Eruca sativa*.

Impotence, erectile dysfunction and decrease in libido are generally regarded self-destructive to many males and the cause of the dulling or breaking of family relations. Various causes underlie the decrease or loss of libido (impotence or erectile dysfunction). These may be psychological such as stress, anxiety, depression and dislikeness of a partner (Low et al., 2006; Shiri et al., 2007), or may be disease-linked as observed in patients with hyperprolactinemia, diabetes mellitus, hypertension, testosterone deficiency and alcoholism. Erectile dysfunction can also occur following a decrease in the availability of the vasodilator and the penile blood flow enhancer nitric oxide and its secondary messenger cyclic GMP (cyclic guanosine monophosphate) (Porst et al., 2003). Furthermore, a local increase in the production of oxygen free radicals or lipid peroxides can lead to penile blood vessels constriction with the consequent reduction in penile blood flow. Thus, this paper investigated the sexual stimulant actions of ginger (*Z. officinale*) and garden rocket (*E. sativa*) and attempted to unravel the molecular mechanisms that underlie their beneficial actions.

Materials and Methods

Chemicals

Enzyme-linked immunosorbent assay (ELISA) kits for determination of cyclic GMP and dihydrotestosterone were purchased from Wuhan El Aab Science co. Ltd., Wuhan, China. 27-Gauge needles (27 G × 0.5 inch) were purchased from Shinwoo Corporation, S. Korea. Kit Kath cannulas (0.8 × 25 mm) were purchased from Hindustan Syringes & Medical Devices Ltd, Faridabad, India). Other chemicals such as sildenafil, papaverine, urethane, ℓ-arginine, Krebs' solution components, oestradiolvalerate, norgestrel, perchloric acid, and heparin were purchased from well-known International companies such as Sigma-Aldrich and BDH.

Plant

The materials ginger (*Z. officinale* Rosocoe) and garden rocket seeds (*E. sativa* Mill.) were purchased from the local market (Riyadh, Kingdom of Saudi Arabia). Both plants, 2,500 g each, were powdered and extracted with 95% ethanol and chloroform. The solvents were evaporated *in vacuo* under reduced pressure to yield 127.1, 107.5, 64 and 470 g, respectively. The chloroform extract of ginger and the ethanol extract of garden rocket seeds were found active. 37.2 g of the active chloroform extract of ginger was partitioned between *n*-hexane and acetonitrile (presaturated with each other) to afford *n*-hexane fraction (16.1 g) and acetonitrile fraction (19 g). 40 g of the active ethanol extract of *E. sativa* seeds were partitioned between *n*-hexane and acetonitrile presaturated with each other to give *n*-hexane fraction (25.4 g) and acetonitrile fraction (10.6 g). The *n*-hexane fractions of ginger and *E. sativa* and the acetonitrile fraction of *E. sativa* were found active.

Animals

The animals used in this study were adult male and female Wistar albino rats. The males' weight was 300 ± 5 g and the females' weight was 220 ± 5 g. All animals were provided with standard chow diet – supplied by Silo and Flour Mills Organization, Feed Mill, Riyadh, Saudi Arabia and boiled and cooled tap water *ad libitum*. All animals were housed at a temperature of 22 ± 1°C and a relative humidity of 50 ± 5%. The light-dark cycle was 12 h each. The animals' treatment was conducted in accordance with the Guide for the Care and Use of Laboratory Animals. The protocol of the current studies was approved by the Ethics Committee of the College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Preparation of the female rats for participation in the experiments

Female Wistar rat at random were made receptive to male partners by bringing them into forced oestrus via the sequential treatment with oestradiol valerate (1.5 mg/kg/day i.p.) for 2 days followed by norgestrel 0.5 mg/kg (i.p.) 6 h before exposing them to the drug-treated male partners.

Preparation of male rats for participation in the behavioral sexual experiments

Male rats were divided into several groups (N = 6 animals per group). One group served as a control. Groups 2 and 3 were injected with sildenafil citrate (dissolved in water) at doses of 25 and 50 mg/kg (i.p.), respectively one hour before their exposure to receptive females. Other groups were injected with 0.25, 0.5 and 1 g/kg doses of ginger or *E. sativa* extract or fraction. All injections were made one hour before exposure of the males to the receptive females. All extracts were suspended in 0.25% sodium carboxy methyl cellulose and vortexed.

Testing of the sexual copulatory behavior

The procedure used for testing the rat's sexual copulatory behavior was adopted and modified from the methods described by (Benassi-Benelli et al., 1979; Hart et al., 1983; Hellegaart and Ahlenius, 1998). In brief: to start the experiment each receptive female and one treated or control male rat were placed in a locally made rectangular Perspex box (60 × 40 × 30 cm). The floor of the arena box was covered with sawdust to a depth of 3 to 5 cm. The box bears 1 cm^2 holes (perforations on the sides and top cover) to

provide adequate aeration. The boxes were placed in an isolated quiet lab (22 ± 2°C, and relative humidity of 55 ± 5%). Each box was monitored by an infra-red video camera positioned at a site that monitors every inch of the box. The camera was purchased from (Maximum Technical Trading Est., Riyadh, Kingdom of Saudi Arabia). All of the experiments started at 9:00 p.m. and continued for 8 h till the next morning. All lights were put off following the start of the experiment. At the end of the monitoring time, CD-recordings of the registered videos were made and analyzed hour-by-hour for 8 h following exposure of treated males to the receptive females for the following male rats' sexual behaviors towards the receptive females: (1) sniffing of vagina; (2) kissing; (3) licking of penis; (4) body grooming; (5) attempt to mount the female; (6) successful mounting and ejaculation. The mean ± SE mean of each of the above parameters per treatment group during the whole first 4 h was compared with that of the control group and those treated with sildenafil. The index of sexual stimulation was calculated as the sum of the number of licking of the penis and the attempts to mount the female per unit time. Successful mountings per unit time were compared separately.

Determination of the concentration of cyclic GMP in the rat's corpus cavernosum

The method used for preparation of the rat's corpus cavernosum incubation medium *in vitro* for determination of the intracellular level of basal and drug stimulated cyclic GMP was adopted and modified from the methods described by Jeremy et al. (1997), Nadackal (2010), Lin et al. (2002), El-Thaher et al. (2002), Seidler et al. (2002), Cirino et al. (2003), Bivalacqua et al. (2004), Matsumoto et al. (2005) and Yang et al. (2008). Aliquots of the incubation media were then assayed for cyclic GMP as directed by the instructions accompanying the ELISA Kit provided by the providing Company Wuhan El Aab Science Co., Ltd., China. The cyclic GMP content was quantified as ng/mg wet tissue.

In brief, the procedure was as follows: Male Wistar rats (300 to 350 g body weight) were anaesthetized with urethane (1.25 g/kg i.p. using 25% aqueous solution w/v). Each animal was placed on its back and its lower limbs fixed with an adhesive tape. The skin overlying the penis was incised. The ischiocaveronus muscle overlying the penile crura was removed partially. Then the corpus spongiosum was removed. All epidermal and connective tissues were removed to clear the penis. The site at which the right and left corpora communicate below the glans penis was located. The two corpora cavernosa were cut longitudinally and placed in cold Krebs' solution (4°C) in a petri dish. The urethra was dissected out and discarded. The longitudinally-cut corpora cavernosa from one rat was placed in a 10 ml glass beaker covered with 5 ml Krebs' solution and incubated at 37°C for 30 min to allow the tissues to recover from the preparative handling. They were then removed, plotted dry on a piece of filter paper and weighed. They were then placed in a 10 ml beaker and chopped finely using a small sharp scissors. To each (weight = 130 to150 mg), 2 corpora cavernosa in the control group 1.05 ml of Krebs' solution, 0.2 ml of 0.25% sodium carboxy methyl cellulose and 0.25 ml of L-arginine (14% aqueous solution) to give a final concentration of 10 µM were added. Thereafter each beaker was incubated for 30 min at 37°C.

To each, 2 corpora cavernosa were cut in the test groups 1.05 ml of Krebs' solution, 0.25 ml of L-arginine (14% aqueous solution w/v) to give a final concentration in the incubation medium equivalent to 10 µM, 0.1 or 0.2 ml of the test fractional extract or the standard sildenafil (33.3 or 66.6 mg sildenafil citrate%) to give a final concentration of 50 or 100 µM or 0.1 or 0.2 ml of papaverine hydrochloride in water (7.5 mg w/v%) to give a final concentration of 50 or 100 µM in the incubation medium were added. The final volume in all incubation media was completed to 1.5 ml using Krebs' solution. The contents of the beakers were incubated for 30 min at 37°C. Following the incubation period, the reaction in each beaker was terminated by the addition of 0.25 ml of 0.5 M perchloric acid in water. Then the contents of each beaker were transferred to a transparent 10 ml centrifuge plastic tubes. 0.25 ml Krebs' solution was added to each beaker mixed to wash all the remainders of the incubation medium and added to the contents of the respective centrifuge tube. The contents of the tubes were then homogenized for 2 min and then centrifuged at (4,000 rpm) for 10 min at 4°C. The supernatants were then re-centrifuged as above and then transferred to 3 ml plastic containers.

To each container, 0.3 ml of 1.25 M trisodium citrate were added to neutralize the acidic supernatants. All supernatant tubes were dipped partially into an ice bath and assayed immediately for the cyclic guanosine monophosphate cyclic (GMP) content. This was performed using ELISA Kits provided by Wuhan El Aab Science Co. Ltd., A 1710, Guungguguoji, East Lake Hi-Tech. Development Zone, Wuhan 430079, China. The cyclic GMP content of each supernatant was then quantified as ng/mg wet weight tissue following the kits instructions.

Determination of dihydrotestosterone in the plasma

To determine the influence of the fractional test extracts or the standard drugs sildenafil and papaverine, the following procedure was used. Male Wistar rats were divided into 9 groups (N = 7 animals per group). One group was used as a control. This was injected with the vehicle 0.25% sodium carboxymethylcellulose (i.p.). The others were injected (i.p.) with sildenafil (25 and 50 mg/kg), papaverine (50 and 100 mg/kg, ginger hexane fraction (0.25 and 0.5 g/kg), and *E. sativa* acetonitrile fraction (0.25 and 0.5 g/kg). Two hours later, corresponding to the time of maximum sexual stimulation as observed in the behavioral studies, all of the animals were anaesthetized with diethyl ether and 4.5 ml blood were collected from each animal using cardiac puncture. The blood was placed in centrifuge tubes containing each 0.5 ml of 3.6% trisodium citrate aqueous solution. The blood was mixed with the citrate and then centrifuged at 1,800 rpm for 10 min to obtain the plasma. The latter was aspirated and each placed in 3 ml glass bottles. All of the bottles were dipped partially in an ice bath and assayed for the content of dihydrotestosterone following the instructions of the ELISA Kits provided by Wuhan El Aab Science Co. Ltd., China.

Effect of the test extracts and the standard drugs on the intracavernosal pressure of rats

The method used to investigate the effect of the extracts and standard drugs on the intracavernosal pressure were adopted and modified from the methods described by Escrig et al. (1999), Bivalacqua et al. (2000), Mcauley et al. (2001), Giuliano et al. (2003), Xiao et al. (2010) and Woo et al. (2011).

In brief, male Wistar rats (350 to 400 g, body weight) were anaesthetized with aqueous urethane (25% w/v) (1.25 g/kg, i.p.). Each was placed on its back on a thermo regulated surgical table and secured in the supine position. The rectal body temperature was maintained at 37°C with a water jacketed heating blanket. The animals were allowed to breathe normal room air. The skin covering the penile glans and shaft was removed using a fine scissors and forceps. The two corpora cavernosa were identified.

To measure the intracavernosal pressure, a 27-gauge needle (27 G × ½ inch) (Shinwoo Corporation, Korea) fitted to one side of a 3-way stopcock was inserted into either the left or right corpus cavernosum at mid length of the penile shaft with the tip of the needle pointing towards the base of penis. The stopcock was connected to a pressure transducer (ITT Statham) to monitor the intracavernosal pressure. The transducer was connected to a Physiograph (Narco-Biosystems, USA) to record the changes in the intracavernosal pressure. The magnitudes of the changes in the pressure were quantified by the calibration system built-in the Physiograph (NarcoBisystems, USA). The needle, the stopcock and the transducer were all filled with heparinized saline 250 U/ml to prevent clotting of blood. When required, drugs were injected intracavernosally via one limb of the 3-way stopcock. When studying the effects of plant extracts the drugs were injected intraperitoneally. The effects of pure compounds were also studied following intravenous injections. For this purpose the right or left external jugular vein was cannulated using Kit Kath intravenous canulae (0.8 × 25 mm fitted with 22 G × 1 inch needles, (Hindustan Syringes and Medical Devices Ltd., 174, 178/25 Ballabgarh, Faridabad, India, 121004). Intravenously or intracavernosally – administered drugs were injected in a maximum volume of 0.2 ml and flushed-in with a similar volume of saline. Each animal was used to test one extract or drug. The interval between the doses of a single drug or extract was one hour.

Composition of Krebs' solution in (mM)

NaCl 118; KCl 4.7; $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11 (pH 7.2 to 7.4) (Lin et al., 2002; Cirinjo et al., 2003).

Statistical analysis

All results were expressed as mean ± SE mean with N = number of experiments performed for each dose used. Significant differences between the control and various treatments were performed using one-way analysis of variance (ANOVA). The least significant difference (LSD) test was used for comparison between each two groups. When ANOVA manifested a significant difference, the Dunnett's or Student-Newman Keuls test was applied. $P < 0.05$ was considered as significant. All statistical analyses were processed through the statistical package for the social sciences (SPSS) version 13 for Windows (SPSS Inc. Chicago).

Results

The beginning of this study started by investigating the effects of the total alcoholic and chloroformic extracts of ginger (*Z. officinale*) and garden rocket (*E. sativa*) extracts in doses of (0.25 to 2 g/kg, i.p.) on the sexual behavior of male rats. These experiments revealed that sexual stimulant effect of ginger was confined to the chloroformic extract whereas that of garden rocket was confined to the alcoholic extract. Each of these extracts was then studied at two dose levels (0.5 and 1 g/kg, i.p.) into male rats. Thereafter, each extract was partitioned between hexane and acetonitrile solvents. Following evaporation of the solvents under vacuum, each fraction was then tested on the sexual behavior of male rats at doses of (0.25 and 0.5 g/kg, i.p.). The results revealed that the activity of ginger was confined to the hexane fraction whereas that of garden rocket was divided between the two fractions with the acetonitrile fraction having 1.5 to 2 times the activity of the hexane fraction. Thus, the hexane fraction of ginger and the hexane and acetonitrile fractions of garden rocket were used to investigate their influence on the behavioral sexual activity, the effects on cyclic GMP levels within the corpora cavernosa, effect on plasma dihydrotestosterone level and on the intracavernosal pressure.

Effects of ginger chloroformic and *Eruca sativa* alcoholic extracts on the sexual behavior of male rats

Treatment of male rats with the chloroformic extract of ginger (*Z. officinale*) in dose of (0.5 and 1 g/kg, i.p.) one hour before exposure to receptive females or with sildenafil in doses of (25 and 50 mg/kg, i.p.) induced dose-dependent increase in the various sexual activity parameters measured compared with vehicle-treated controls. The highest doses tested of the extracts and sildenafil induced significant increase ($P < 0.01$, N = 6). Table 1 depicts the effects of the extracts at a dose of (1 g/kg, i.p.) and those of sildenafil (50 mg/kg, i.p.) compared with the control animals during 4 h observation period.

As shown in Table 1, both of ginger and garden rocket together with sildenafil induced significant increase in the three major sexual activity parameters namely licking of penis, attempt to mount the female and the successful mounting and ejaculation. The effects of *E. sativa* and sildenafil were almost similar regarding the first two parameters but sildenafil treated animals were more active ($P < 0.05$) – almost > 60% more active – regarding the successful mounting and ejaculation parameter. Both of sildenafil and *E. sativa* were more than twice as active as *Z. officinale*.

With regard to the sexual stimulation index during the whole 4 h observation period, the calculated values were: 39.9 ± 3.6, 120 ± 2.5, 355 ± 4.8 and 401 ± 30.1, for the control, ginger, *E. sativa* and sildenafil, respectively. Here again, the differences between the various treatments and the control were significant ($P < 0.01$, N = 6). The score of *E. sativa* was almost similar to that of sildenafil ($P > 0.05$).

Effect of ginger hexane fraction and *E. sativa* hexane and acetonitrile fractions on the sexual behavior of male rats

Treatment of male rats one hour before exposure to receptive female partners with the ginger hexane fraction

Table 1. Effects of *Z. officinale* chloroformic and *E. sativa* alcoholic extracts and sildenafil on the male rat sexual activity parameters during a 4 h observation period following their exposure to receptive females.

Treatment / Sexual parameter	Count score/4 h			
	Control	*Z. officinale* (1 g/kg)	*E. sativa* (1 g/kg)	Sildenafil (50 mg/kg)
Licking of penis	21.3±0.6	63±1.5*	175±5.1**	212±7.5*
Attempt to mount female	18.6±1.1	67±2.7*	180±6.9**	189±9.6**
Sniffing of vagina	15.8±5.1	5±0.1	24±2.7	16.5±4.2
Kissing	8.8±1.7	10±1.7	30±2.3	25±5.1
Body grooming	7.1±2.7	9±0.7	15±0.7	21±4.2
Successful mounting and ejaculation	1.6±0.6	7±0.4*	11±1**	18±1.7**

*$P < 0.05$, N = 6 compared with control. **$P < 0.001$, N = 6 compared with control.

Table 2. Effect of the chloroformic/hexane fraction of ginger, the alcoholic/hexane and the alcoholic/acetonitrile fractions of *E. sativa* on the male rats sexual parameters during the 4-hours observation period following their exposure to the receptive female partners.

Treatment (dose) / Sexual parameter	Count score/4 h			
	Control	*Z. officinale* (hexane) (0.5 g/kg)	*E. sativa* (hexane) (0.5 g/kg)	*E. sativa* (acetonitrile) (0.5 g/kg)
Licking of penis	27.6±1.8	57±3.9*	71±5.1*	117±1.5**
Attempt to mount female	26.2±2.9	59±1.7*	83±5.1**	136±3.9**
Sniffing of vagina	15.2±0.3	25±2.1	20±1.3	17.5±0.7
Kissing	13.1±2.1	11±0.9	16±0.4	21±0.3
Body grooming	11.4±0.9	34±2.6	15±0.6	21±0.7
Successful mounting and ejaculation	2.6±0.5	19±0.5**	6±0.3*	13±0.6**

*$P < 0.05$, N = 6compared with control. **$P < 0.01$, N = 6 compared with control.

at doses of (0.25 and 0.5 g/kg, i.p.) or with the hexane and acetonitrile fractions of *E. sativa* at similar doses induced dose-dependent increases in the sexual activity parameters during the 4 h observation period. Significant values compared with the control were observed following treatment of the animals with the higher doses. Table 2 depicts the cumulative results from these studies. As shown in this table, the hexane fractions of both ginger and garden rocket together with the acetonitrile fraction of the latter all enhanced the various sexual parameters with significant differences ($P < 0.05$ to $P < 0.01$) regarding the most prominent sexual activity parameters – namely licking of the penis, attempt to mount the female and the successful mounting and ejaculation.

It should be noted that there was no significant difference in the count score of the total chloroformic and the chloroformic/hexane fraction of ginger regarding the first two parameters. However, the hexane fraction exerted significantly more successful mounting and ejaculation – almost more than twice ($P < 0.01$, N = 6) compared with the total chloroform extract. It is interesting to note that in case of *E. sativa*, the partition of the alcoholic extract between hexane and acetonitrile resulted in distributing the sexual stimulant effects between the two fractions albeit with significantly more increase, almost double, ($P < 0.05$) with regard to the successful mounting and ejaculation parameter in the acetonitrile fraction compared with the hexane fraction. Furthermore, the count scores of the acetonitrile fraction of garden rocket regarding the licking of the penis and attempts to mount female were significantly greater than those of the hexane fraction ($P < 0.05$, N = 6).

Regarding the sexual stimulation index during the whole 4 hours observation period, the calculated values were 53.8 ± 2.7, 116 ± 3.8, 154 ± 5.1 and 253 ± 2.7 for the control, ginger and *E. sativa* hexane fractions and *E. sativa* acetonitrile fraction. Here again, all of the indices were significant compared with the control value ($P < 0.05$, N = 6).

Table 3. Effect of the hexane fractions of ginger and *E. sativa*, the acetonitrile fraction of *E. sativa*, sildenafil and papaverine on cyclic GMP content in the rat corpus cavernosum.

Treatment	Cyclic GMP (ng/mg wet tissue) in the rat corpus cavernosum	% Increase
Control	0.94±0.07	-
Ginger (hexane fraction) (0.2 g)	2.81±0.19**	198.9±9.4
E. sativa (hexane fraction)	1.36±0.03*	44.7±9.7
E. sativa (Acetonitrile fraction) (0.2 g)	2.65±0.14**	181.9±11.3
Papaverine (100 µM)	1.04±0.03	10.6±3.1
Sildenafil (100 µM)	3.66±0.19**	289.3±7.5

*P < 0.05, N = 7, compared with control. **P < 0.001, N = 7, compared with control.

Table 4. Effect of the hexane and acetonitrile fractions of ginger and garden rocket and sildenafil on dihydrotestosterone plasma level in male rats.

Treatment (dose/kg, i.p.)	Concentration of dihydrotestosterone in plasma (pmole/ml)	% Change
Control	1.12±0.08	-
Ginger (hexane fraction) (0.5 g)	1.78±0.04*	↑58.9±0.6
E. sativa (hexane) (0.5 g)	1.44±0.08*	↑25.9±1.8
E. sativa (Acetonitrile) (0.5 g)	1.21±0.05	↑8±0.9
Sildenafil (50 mg)	1.06±0.1	↓5.3±8.1

*P < 0.05, N = 7, compared with control.

Effect of the active hexane and acetonitrile fractions on cyclic GMP content in the rat corpora cavernosa

Incubation of normal rat corpus cavernosa *in vitro* under the conditions described in this study revealed an excellent quantifiable content of cyclic GMP, almost 1 ng/mg wet weight tissue. Incubation of the tissue in presence of the chloroform/hexane fraction of either ginger or garden rocket, the acetonitrile fraction of garden rocket alcoholic extract and sildenafil but not with papaverine resulted in significant increases in the cyclic GMP content (P < 0.05, N = 7).

The increase was dose-dependent and reached significant levels following incubation of the tissue with the highest dose tested. The presence of papaverine hydrochloride in final concentrations of 50 and 100 µM did not increase the content of cyclic GMP (P > 0.05, n = 7).

The cumulative results are shown in Table 3. One thing to note about the *E. sativa* fractions is that here again as in the behavioral study, the acetonitrile fraction produced significantly more increases in the content of cyclic GMP compared with the hexane fraction (P < 0.05, N = 7). Furthermore, ginger hexane fraction was as active as the acetonitrile fraction of *E. sativa,* albeit it was less active than the latter fraction in the behavioral study.

Effect of the active hexane fractions of ginger and *E. sativa*, the acetonitrile fraction of *E. sativa* and sildenafil on dihydrotestosterone plasma level

The basal level of dihydrotestosterone, the active metabolite of testosterone in the rat's plasma was found to be 1.12 ± 0.08 pmole/ml plasma (N = 7). Treatment of male rats with ginger hexane fraction, *E. sativa* hexane fraction in doses of (0.25 or 0.5 g/kg, i.p.) or sildenafil (25 or 50 mg/kg, i.p.) resulted in significant increases only with the higher doses of both *E. sativa* and ginger hexane fractions (P < 0.05, N = 7). Table 4 depicts the cumulative results using the higher doses.

Effect of the hexane fractions of ginger and *E. sativa*, the acetonitrile fraction of *E. sativa*, sildenafil and papaverine on the intracavernosal pressure in rats

Intracavernosal injection of sildenafil (1 and 2 mg), papaverine (2 and 6 mg) into anaesthetized rats produced dose-dependent increases in the intra-cavernosal pressure. Similarly, administration of 25 and 50 mg sildenafil/kg (i.v.) or 200 and 400 mg papaverine/kg (i.p.) induced dose-dependent increases in the intracavernosal pressure. Administration of the hexane

Table 5. Effect of the hexane fractions of ginger and *E. sativa*, the acetonitrile fraction of *E. sativa*, sildenafil and papaverine on the anaesthetized rat intracavernosal pressure.

Treatment (dose)/kg	Route of administration	Increase in the intracavernosal pressure (mmHg)
Ginger (hexane extract) (0.5 g)	i.p	4.2±0.3
E. sativa (hexane extract) (0.5 g)	i.p	2.6±0.1
E. sativa (acetonitrile extract) (0.5 g)	i.p	3.7±0.3
Papaverine hydrochloride (0.2 g)	i.p	3.3±0.5
Sildenafil (2 mg)	Intracavernosally	4.2±0.9

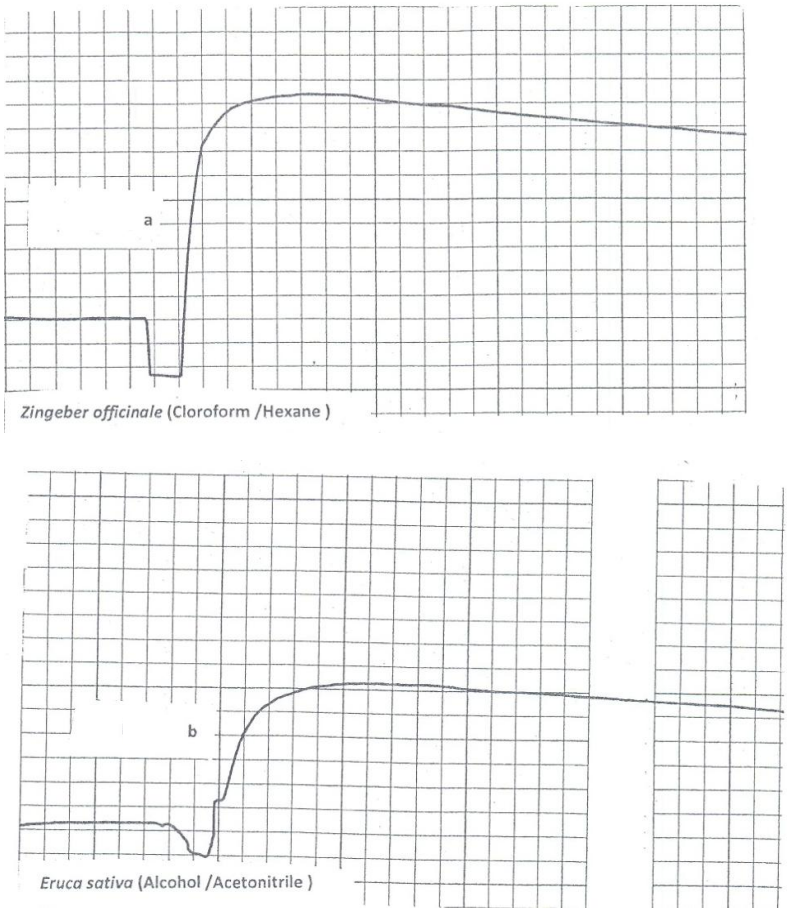

Figure 1. (a) Effect of the hexane axtract of ginger on the rat intracavernosal pressure at doses of (0.5 g/kg i.p); (b) effect of the acetonitrile extract of E. sativa on the rat intracavernosal pressure at doses of (0.5 g/kg i.p).

fractions of ginger and *E. sativa*, and the acetonitrile fraction of *E. sativa* in doses of 0.25 and 0.5 g/kg, i.p. induced dose-dependent increases in the intracavernosal pressure following a delay of 10 to 15 min. Figures 1a, b, 2a and b show representative effects from such studies. Table 5 summarizes the cumulative increases in the intracavernosal pressures (N = 4).

DISCUSSION

The results of this study clearly demonstrated the

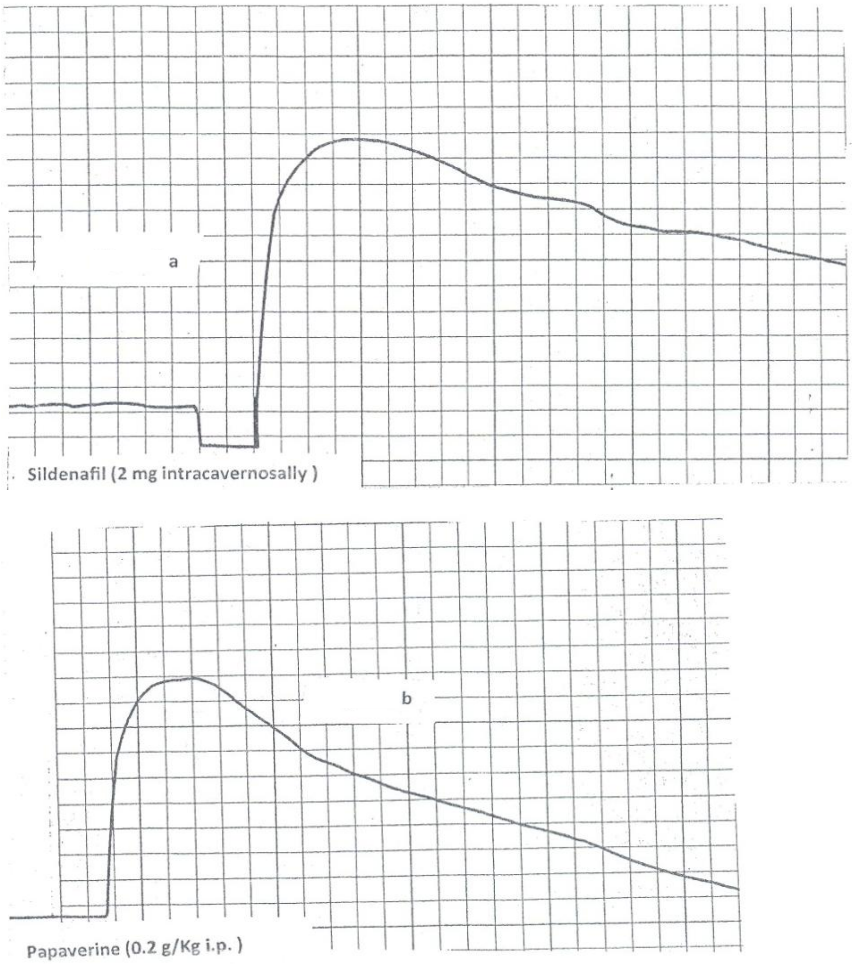

Figure 2. (a) Effect of sidenafil citrate (2 mg intracavernosally) in the intracavernosal pressure of the rat, (b) effect of papaverine hydrochloride (200 mg/kg, i.p) in the intracavernosal pressure on the rat.

inherent ability of the chloroformic/hexane fraction of ginger (*Z. officinale* Roscoe), the alcoholic/hexane and the alcoholic/acetonitrile fractions of *E. sativa* to significantly enhance the sexual activity of male rats in both behavioural parameters, as revealed by the significant increases in the sexual activity index and in the functional studies as revealed by the increases in the intracavernosal pressure, a reliable positive index of erectile function. In most of the parameters tested, *E. sativa* acetonitrile fraction seemed to be more potent than ginger hexane extract but less active than sildenafil. It should also be noted that ginger hexane extract although had a lower sexual index than both sildenafil and *E. sativa* fractions, yet it was as effective as sildenafil in stimulating successful mounting and ejaculation.

The biochemical studies performed to shed light on the mechanisms of action of the observed stimulant effects revealed the ability of both herbs to significantly stimulate the production or availability of cavernosal cyclic GMP content. Furthermore, the hexane fractions of both herbs significantly elevated the plasma level of dihydrotestosterone, the active metabolite of the established libido stimulant testosterone. An increase in the intracellular cavernosal cyclic GMP content is usually linked with cavernosal smooth muscles relaxation, with an increase in penile blood flow and consequent erection (Porst et al., 2003). A supplementary mechanism for both herbs comes from the reported antioxidant action. For instance *E. sativa* seed constituents, glucoerucin (Barillari et al., 2005) and polyphenols (Sarwar et al., 2007) are shown to possess such activity (Badee et al., 2003a; Barillari et al., 2005; Kim and Ishii, 2006; Sarwar et al., 2007).

A similar antioxidant effect was reported for ginger constituents 6-gingerol (Aeschbach et al., 1994; Katsunari et al., 2007; Sekiwa et al., 2000; Siddaraju and

Dharmesh, 2007) and zingerone (Aeschbach et al., 1994). Such antioxidant action may help to combat the accumulation of free oxygen radicals and lipid peroxides that are known to constrict blood vessels and hence their antagonism can enhance penile blood flow with the consequent erection. Furthermore, an inherent cholinergic activity (Ghayur et al., 2007) possibly through muscarinic M_2 autoreceptor blockade coupled with the ability to block calcium channels (Ghayur and Gilani, 2005) in ginger extract may act to increase penile blood flow and relaxation of the corpora cavernosa with consequent erection. A similar cholinergic action was also noted for *E. sativa* oil (contained in the hexane extract) via inhibition of cholinesterase (Boga et al., 2011).

In this study, no attempt was made to pin-point the actual ginger or *E. sativa* candidate(s) responsible for the observed aphrodisiac actions and the enhancement of the sexual activity. Ginger is known to contain various constituents such as 6-, 8- and 10-gingerol, 6-shogaol (Schwertner and Rios, 2008), zingerone (Aeschbach et al., 1994), and various glucosides such as gingerdil (Sekiwa et al., 2000). Thus, any of these constituents singly or in concert with others may be responsible for the observed aphrodisiac actions. However, 6-gingerol may be excluded from the above conjecture as it is reported to inhibit the synthesis of the penile vasodilator nitric oxide albeit in macrophages (Radi et al., 2000) and in microglial cell line (Jung et al., 2009).

On the other hand, *E. sativa* seeds are known to contain various constituents (the major ones being desulfoglucosinolates) (Adhikari et al., 1989; Kim and Ishii, 2006; Sarwar et al., 2007; Shen and Xu, 2007). These included glucoerucin (4-methylthiobutyl glucosinate) (Adhikari et al., 1989; Kanya and Urs, 1989; Barillari et al., 2005; Sarwar et al., 2007; Nazif et al., 2010), glucoraphanin, glucobrassin, dimerico 4-mercaptobutylglucosinate (Kim and Ishii, 2006), gluconapin (Adhikari et al., 1989), Singrin, gluco-brassicanapin (Adhikari et al., 1989) and glucoiberin (Nazif et al., 2010). In addition to these, the hexane fraction is expected to contain a fixed oil which is rich in erucic acid (Popov and Mazhdrakov, 1958; Gad et al., 1965, Khan et al., 1984; Nazif et al., 2010; Gulfraz et al., 2011) and a volatile oil that is rich in isothionates (Hals and Gram, 1909; Mahran et al.,1992; Badee et al., 2003a, b) such as 4-methylthiobutyl isothiocyanate (Badee et al., 2003a, b) and 4-methylthio pentanonitrile (Mahran et al., 1992). Thus, the actual aphrodisiac candidate may be any of the above or a combination of them. Only further experiments can pin-point the actual candidate.

On a broad basis, the results of this study point to the potential inherent sexual stimulant action of the chloroform/hexane and acetonitrile fraction of *E. sativa* and the alcoholic/hexane fraction of ginger (*Z. officinale*) in rats to an extent that approaches that observed with sildenafil. It is hoped that further experimental and clinical studies may pave the way towards introduction of these two herbs as new competitors for the available phosphodiesterase 5 inhibitors in the treatment of decreased libido, erectile dysfunction and impotence in males.

ACKNOWLEDGEMENTS

We thank Mr. Mokhair, Mohammed and Malik Saud for their appreciable technical help. The work was financially supported by NPST program by King Saud University, Project # (08-MED523-2).

ABBREVIATIONS

I.CV, Intracavernosal pressure; **C/H,** hexane fraction of the chloroformic extract; **A/Ac,** acetonitrile fraction of the alcoholic extract.

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