Molecular mechanisms that underlie the sexual stimulant actions of *Avicennia marina* (Forssk.) Vierh. and *Crocus sativus* L.

Adnan Jathlan Al-Rehaily*, Tawfeq Abdullah Alhowiriny, Kamal EH El Tahir, Areej Mohammad Al-Taweel and Shagufta Perveen
Departments of Pharmacognosy and Pharmacology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Abstract: The effects of extracts and sub-fractions of *Avicennia marina*, *Crocus sativus* and sildenafil on the sexual behavior of male rats and their effects on the intracavernosal pressure (I.CV), intracavernosal cyclic 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 sub-fraction in case of *A. marina* was the hexane fraction of the chloroform extracts (C/H) whereas that of *C. sativus* was the hexane fraction of the alcoholic extract (A/H). (C/H), (A/H) and sildenafil significantly increased the total sexual stimulation index from 53.8±2.7 (control) to 406±7.8, 225±4 and 401±30.1, respectively ($P<0.001$, N=6). They significantly increased the index of successful mounting and ejaculation from 2.6±0.5 (control) to 40±2.7, 21±2.3 and 18±1.7, respectively ($P<0.01$, N=6). They significantly increased the cyclic GMP level from 0.94±0.07 (control) to 3.1±0.13, 1.59±0.11 and 3.66±0.19 ng/mg wet tissue, respectively ($P<0.05$, N=7). They did not affect dihydrotestosterone plasma level. (C/H), (A/H) and sildenafil increased the (I.CV) pressure by 4.8±0.3, 1.4±0.8 and 4.2±0.9 mmHg. The (C/H) seemed to be more active than sildenafil and twice active than (A/H). Both extracts and sildenafil acted via an increase in cyclic GMP.

Keywords: *Avicennia marina*, *Crocus sativus*, sexual behavior, intracavernosal pressure, cyclic GMP, 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 (e.g. snakes), bees' products (honey and propolis) and even inorganic minerals e.g. 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 (Chopra *et al.,* 1956; 1966; Said *et al.,* 1996). The list of such plants is long but what strikes one is the continuous repetition of certain herbs in all of the investigated cultures. Some of these included ginger rhizomes from *Zingiber officinale*, saffron stigmas from (*Crocus sativus*) garden rocket 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 them of two herbs namely *Avicennia marina* and saffron.

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 (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 (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 *A. marina* and *C. sativus* and attempted to unravel the molecular mechanisms that underlie their beneficial actions.

MATERIALS AND METHODS

Chemicals

ELISA kits for determination of cyclic GMP and dihydrotestosterone were purchased from Wuhan El Aab Science Co., Ltd., Wuhan, China. Needles and cannulae 27-Gauge needles (27G×0.5 inch) were purchased from Shinwoo Corporation, S. Korea. Kit Kath cannulae (0.8 × 25 mm) were purchased from Hindustan Syringes and Medical Devices Ltd, Faridabad, India). Other chemicals such as sildenafil, papaverine, urethane, ℓ-arginine Krebs'

**Corresponding author:* e-mail: ajalreha@ksu.edu.sa

solution components, oestradiolvalerate, norgestrel, perchloric acid, heparin were purchased from well-known International companies such as Sigma-Aldrich and BDH.

Animals

The animals used in this study were adult male and female Wistar albino rats. The males' weight was 300±5g and the females' weight was 220±5g. 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 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, Kingdom of Saudi Arabia.

Plant material and extraction procedure

The material Saffron (*C. sativa* L.) was purchased from local market. The aerial parts of *A. marina* (Forssk.) Vierh. were collected on 12 March, 2011 from Southern coast of the Kingdom of Saudi Arabia. The plant was identified by Dr. Mohammed Yusuf, Taxonomist at the Pharmacognosy department, College of pharmacy, King Saud University (KSU). A voucher specimen (# 15757) was deposited at the herbarium of the College of Pharmacy, KSU. Both plants, 2000g each, were powdered and extracted with 95% ethanol and chloroform at room temperature 25±3°C for 72h. The solvents were evaporated *in vacuo* at 40°C under reduced pressure to yield 135g, 350.0, 55.0g and 93.0g, respectively. The chloroform extract of *A. marina* and the ethanol extract of saffron were found active.

45.0g of the active chloroform extract of *A. marina* was partitioned between *n*-hexane and acetonitrile (presaturated with each other) to afford *n*-hexane fraction (27.0g) and acetonitrile fraction (10.4g) and insoluble fraction (7.2g). 300g of the active ethanol extract of saffron was partitioned between *n*-hexane and acetonitrile presaturated with each other to give *n*-hexane fraction (122.1g) and acetonitrile fraction (18.0g) and insoluble fraction (153.5g). The *n*-hexane fractions of *A. marina* and saffron were found active.

Preparation of the female rats for participation in the experiments

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

Preparation of male rats for participation in the behavioral sexual experiments

In the initial testing 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 doses of *A. marina* or saffron extract or fraction. All injections were made one hour before exposure of the males to the receptive females. All of the extracts were suspended in 0.25% sodium carboxy methyl cellulose and vortexed vigorously.

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 saw-dust to a depth of 3-5 cm. The box bears 1cm^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 and performed at 8-9 p.m. and continued for 7-8 hours 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 the first 4 hours 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±s.e. mean of each of the above parameters per treatment group during the whole 4 hours 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 females 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 (Jermy *et al.,* 1997; Nadackal, 2010; Seidler

et al., 2002; Cirino *et al.,* 2003; Bivalacqua *et al.,* 2004; Matsumoto *et al.,* 2005; Yang *et al.,* 2008).

Aliquots of the incubation media were then assayed for cyclic GMP as directed by the instructions accompanying the ELISA Kits provided by the providing company Wuhan El Aab Science Co., Ltd., China. The cyclic GMP content was quantified as ngcGMP per mg wet weight tissue. In brief, the procedure was as follows:

Nine groups of male Wistar rats (300-350g body weight, N=7 animals per group) were anaesthetized with urethane (1.25g/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) (table 1) in a petri dish. The urethra was dissected out and discarded. The longitudinally-cut corpora cavernosa from one rat were placed in a 10-ml glass beaker, covered with 5 ml Krebs' solution and incubated at 37°C for 30 minutes 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 2 corpora cavernosa (weight=130-150 mg) in the control group 1.05ml of Krebs' solution, 0.2ml of 0.25% sodium carboxy methylcellulose and 0.25ml of L-arginine (14% aqueous solution to give a final concentration of 10μM) were added. Thereafter each beaker was incubated for 30 min at 37°C.

To each cut 2 corpora cavernosa in the test groups 1.05ml of Krebs' solution, 0.25ml of L-arginine (14% aqueous solution w/v) to give a final concentration in the incubation medium equivalent to 10μM, 0.1 or 0.2ml (0.1 or 0.2g) of the test fractional extract or the standards sildenafil (33.3 or 66.6mg sildenafil citrate %) to give a final concentration of 50 or 100μM or 0.1 or 0.2ml of papaverine hydrochloride in water (7.5mg%, w/v) to give a final concentration of 50 or 100μM in the incubation medium were added. The final volume in all incubation media was completed to 1.5ml 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 of 0.5 M perchloric acid in water. Then the contents of each beaker were transferred to a transparent 10-ml centrifuge plastic tubes. 0.25ml 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 minutes, and then centrifuged at (4000 rpm) for 10 minutes at 4°C. The supernatants were then re-centrifuged as above and then transferred to 3ml plastic containers.

To each container 0.3ml of 1.25M 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.

Table 1: Composition of the Krebs' solution

Ingredients	Quantities for 10 litres of
NaCl	69g
KCl 10 per cent	35ml
$MgSO_4$, $7H_2O$ 10 per cent	29ml
KH_2PO_4 10 per cent	16ml
Glucose	20g
$NaHCO_3$	21g
$CaCl_2$ (Molar)	25.2 ml
Aerating gas	O_2 + 5% CO_2

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 100mg/kg), *A. marina* chloroformichexane fraction (0.25 and 0.5g/kg) and saffron (*C. sativus*) alcoholic hexane 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 4.5ml blood were collected from each animal using cardiac puncture. The blood was placed in centrifuge tubes containing each 0.5ml of 3.6% trisodium citrate aqueous solution. The blood was mixed with the citrate and then centrifuged at 1800 rpm for 10 min to obtain the plasma. The latter was aspirated and each placed in 3ml 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 Sci., 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; Woo *et al.,* 2011).

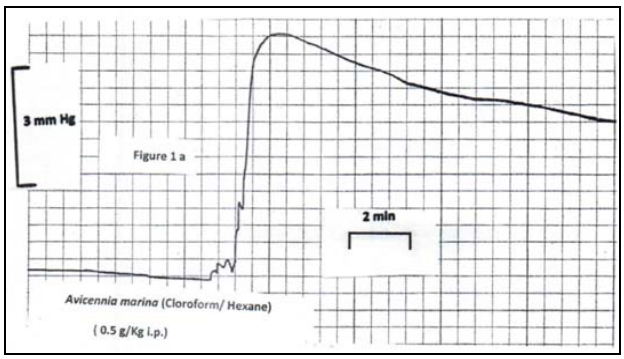

Fig. 1a: Effect of the hexane fraction of A. marina on the intracavernosal pressure in rats

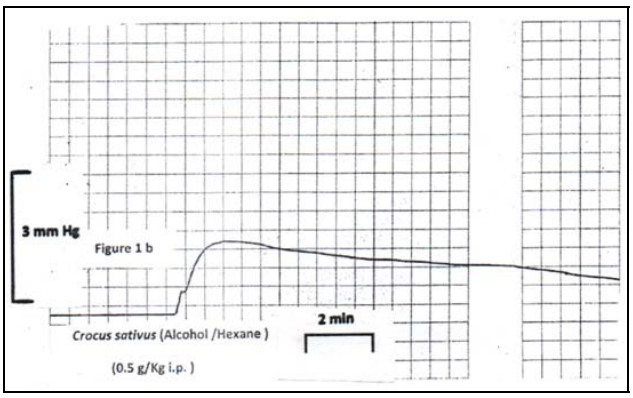

Fig. 1b: Effect of the hexane fraction *C. sativus* on the intracavernosal pressure in rats

Pressure buildup started at 10 min. following the extract administration and reached to maximum after 2 min.

In brief, Male Wistar rats (350-400 g, body weight) were divided into 4 groups (N=4 animals per group) and anaesthetized with aqueous urethane (25% w/v, 1.25g/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 (27G×½ inch) (Shinwoo Corporation, Korea), fitted to a 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 (Narco Biosystems, USA). The needle, the stopcock and the transducer were all filled with heparinized saline 250 U/ml to prevent clotting of blood. Sildenafil was injected intracavernosally via one limb of the 3-way stopcock or (i.v) whereas the extracts or papaverine were injected (i.p).

For the (i.v) injections the right or left external jugular vein was cannulated using Kit Kath intravenous cannulae (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 0.2ml 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. For the extracts and drugs a dose-response curve was first obtained and a sub maximal dose was selected.

STATISTICAL ANALYSIS

All results were expressed as mean ±s.e. 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 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.).

RESULTS

Effects of total alcoholic and chloroform extracts on the sexual behavior of male rats

The first part of this study examined the effect of the total alcoholic and chloroformic extracts in doses of (0.25-2g/kg, i.p.) on the sexual behavior of male rats. These experiments revealed that the sexual stimulant effect of *A. marina* was confined to the chloroformic extract and that of saffron (*C. sativus*) was confined in the alcoholic extracts. Each of these extracts was then studied at two dose levels (0.25 and 0.5g/kg, i.p.). Thereafter each of the active extracts was partitioned between hexane and acetonitrile solvents. Following evaporation of the solvents under vacuum each fraction was tested on the sexual behavior of male rats at doses of (0.25 and 0.5 g/kg, i.p.). The results revealed that in both plants the activity was confined to the hexane fraction. Thus, these

Table 2: Effects of *A. marina* (chloroformic), Saffron (alcoholic) extracts and sildenafil on the male rat sexual activity parameters during 4-hour observation period following their exposure to receptive females

Treatment / Sexual Parameters	Count score / 4 hours			
	Control	*Avicennia* (1 g/kg)	Sildenafil (50 mg/kg)	Saffron (1 g/kg)
Licking of penis	21.3±0.6	108±4**	212±7.5**	44±3.5*
Attempt to mount female	18.6±1.1	105±9**	189±9.6**	47±3*
Sniffing of vagina	15.8±5.1	16.3±1.9	16.5±4.2	14±1.5
Kissing	8.8±2.7	19±2[*]	25±5.1[a]	9±1.2
Body grooming	7.1±2.7	14±1.9[*]	21±4.2[a]	7±0.3
Successful mounting and ejaculation	1.6±0.6	12±1.5**	18±1.7**	6±1**

*P<0.05, N=6 compared with control. **P<0.001, N=6 compared with control. [a]P<0.01, N=6 compared with the control.

Table 3: Effect of *A. marina* and saffron hexane fractions on the male rats sexual parameters during 4-hour observation period following their exposure to receptive female partners

Treatment / (Dose) Sexual Parameters	Count score / 4 hours		
	Control	*Avicennia* (0.5 g/kg)	*Saffron* (0.5 g/kg)
Licking of penis	27.6±1.8	189±10.1**	128±6.1**
Attempt to mount female	26.2±2.9	217±5.3**	97±2.3**
Sniffing of vagina	15.2±0.3	37±3.1*	43±1.6*
Kissing	13.1±2.1	40±3.7*	35±1.9*
Body grooming	11.4±0.9	36±1.1*	33±2.1*
Successful Mounting and Ejaculation	2.6±0.5	40±2.7**	21±2.3**

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

hexane fractions were used to investigate the influence of each plant on the behavioral studies regarding the sexual activity, effect on cyclic GMP levels within the corpora cavernosa, effect on plasma dihydrotestosterone level and on the intracavernosal pressure.

Effects of A. marina chloroformic and saffron alcoholic extracts on the sexual behavior of male rats

Male Wistar rats were divided into 7 groups (N=6 animals per group). Treatment of the animals with chloroformic extract of *A. marina*, the alcoholic extract of saffron (*C. sativus*) in doses of 0.5 and 1g/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-related increases in the sexual activity parameters measured compared with the vehicle-treated controls. Significant level was attained for all treatments at the highest dose tested for the extracts and sildenafil (P<0.05, N=6). Table 2 depicts the effects of 1g extract/kg and the effect of 50mg sildenafil/kg compared with the control during the whole four hour-observation period.

As shown in table 1, both *A.marina* and saffron extracts together with sildenafil induced significant increases in 3 sexual parameters namely: licking of the penis, attempt to mount the female and successful mounting and ejaculation. Furthermore, both *A. marina* and sildenafil also significantly increased the kissing and body grooming (P<0.05 and p<0.01, N=6), respectively compared with the control.

The effect of sildenafil was almost twice that of *A. marina* extract and four times that saffron regarding the licking of the penis and the attempts to mount the female. However, with regard to successful mounting and ejaculation, sildenafil was 1.5 times more active than *A. marina* and 3 times more active than saffron.

With regard to the sexual stimulation index during the whole 4-hour observation period, the calculated values were 39.9±3.6, 213±2.9, 401±30.1 and 91±3.7 for the control, *A. marina*, sildenafil and saffron, respectively. Here again the differences between the various treatments and the control values were significant (P<0.01, N=6).

Effect of A. marina and saffron Hexane fractions on the sexual behavior of male rats

Male Wistar rats were divided into 5 groups (N=6 animals per group). Treatment of the animals with the hexane fractions of *A. marina* and saffron in doses of (0.25 and 0.5g/kg, i.p.) one hour before exposure to receptive female partners induced dose-dependent increases in the sexual activity parameters during the 4-hour observation period. Significant levels were attained only following the administration of the (0.5 g/kg) doses. Table 2 depicts the cumulative results from six experiments of each compared with the vehicle-treated control. As clear from the table the hexane fractions of *A. marina* and saffron significantly enhanced all of the sexual parameters measured (P<0.05-P<0.001, N=6). The count scores

regarding all of the sexual parameters observed was significantly increased in the hexane fractions compared with the parent chloroformic and alcoholic extracts of *A. marina* and saffron, respectively ($P<0.01$, N=6). This enhancement was 2-4 times in case of saffron hexane extract. The most striking finding was observed following administration of *A. marina* hexane extract regarding the successful mounting and the ejaculation parameter which was found to be more than 3 times that of the parent chloroformic extract and more than twice that of sildenafil ($P<0.05$, N = 6).

Regarding the sexual stimulation index during the whole 4-hour observation period, the calculated values were: 53.8±2.7, 406±7.8 and 225±4.6 for the control, *A. marina* and saffron hexane fractions, respectively. Here again the indices of *A. marina* and saffron were significantly high compared with the control ($P<0.001$, N=6). These fractions indices were twice those of the parent chloroform and alcoholic extracts for *A. marina* and saffron, respectively. *A. marina* hexane fraction index obtained following treatment with 0.5g fraction/kg i.p. was almost equivalent to that observed following treatment with sildenafil (50mg/kg, i.p.). (See tables 2 and 3 and the text above).

Effect on cyclic GMP in the corpora cavernosa

Incubation of normal rat corpus cavernosa *in vitro* in the conditions described in this study revealed an excellent content of cyclic GMP reaching almost 1ng/mg wet weight tissue. The presence of sildenafil and the hexane fractions of *A. marina* and saffron but not papaverine induced significant increases in cyclic GMP content that were 3 times the basal content in cases of sildenafil and the *A. marina* extract. Incubation of the tissue with the hexane fractions in quantities of 0.1 and 0.2g/medium or sildenafil final medium concentration of 50 or 100µM increased the content of cyclic GMP in a concentration dependent manner. However, the presence of papaverine in final concentrations of 50 and 100 µM in the medium did not increase the cyclic GMP. table 4 depicts the cumulative results from 7 experiments using 0.2g, 100µM and 100µM in the medium for the extracts, sildenafil and papaverine, respectively.

Effect on dihdrotestosterone plasma level

The basal plasma level of dihydrotestosterone of the male rats used in this study was found to be 1.12±0.08 pmole/ml plasma (N=7). Treatment of male rats with the hexane fractions of *A. marina* and saffron in doses of 0.25 and 0.5g/kg, i.p. or sildenafil 25 and 50mg/kg, i.p., did not affect the basal level of dihydrotesterone to any significant level. Table 5 depicts the cumulative results from 7 experiments using 0.5g/kg for the extracts and 50 mg/kg for sildenafil.

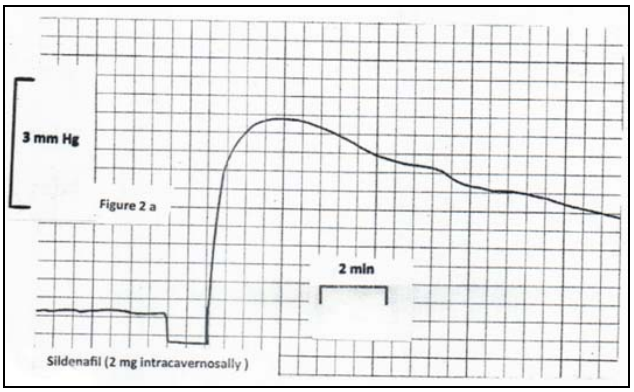

Fig. 2a: Effect of the hexane fraction sildenafil on the intracavernosal pressure in rats
Pressure buildup started at 1 min. following the sildenafil administration and reached to maximum after 2 min.

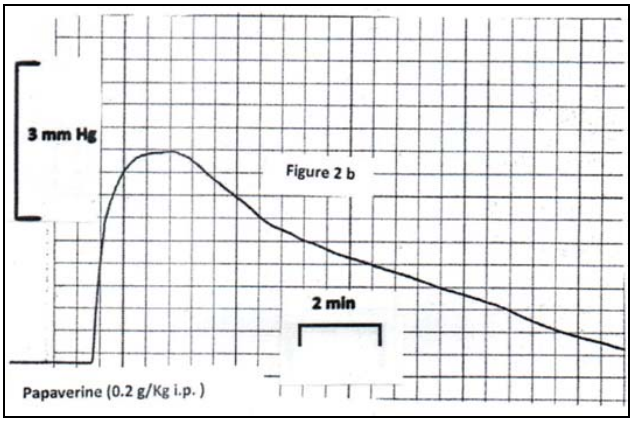

Fig. 2b: Effect of the hexane fraction papaverine on the intracavernosal pressure in rats
Pressure buildup started at 10 min. following the papaverine administration and reached to maximum after 2 min.

Effect of the hexane fractions of A. marina and saffron, sildenafil and papaverine on the intracavernosal pressure in rats

Intracavernosal administration of sildenafil (1 and 2 mg) papaverine (2 and 6 mg) into anaesthetized rats induced dose-dependent increases in the intracavernosal pressure. Similarly administration of 25 and 50mg, sildenafil /kg (i.v.) or 200 and 400mg papaverine /kg (i.p.) induced dose-dependent increases in the intracavernosal pressure. Administration of *A. marina* hexane fraction or saffron hexane fraction in doses of 0.25 and 0.5g/kg intraperitoneally induced dose-dependent increases in the intracavernosal pressure following a delay of 10-15 minutes. The increases were dose-dependent. fig. 1a and b and 2a and b show the effects of some of these results. Table 6 summarizes the increases in the intracavernosal pressure in (mmHg) obtained from such types of experiments (N=4). The increases induced by *Avicennia* extract, papaverine and sildenafil albeit small, yet they were significant compared to a basal level of 3.8±0.7

mmHg ($p<0.05$, N=4). The reported values were the net increases above the basal level.

Table 4: Effect of *A. marina* and saffron hexane fractions, sildenafil and papaverine on cyclic GMP content in the rat corpus cavernosum

Treatment (Final content in the medium)	Cyclic GMP (ng/mg wet tissue) in the rat corpus cavernosum	% Increase
Control	0.94±0.07	—
Avicennia Extract(0.2 g)	3.1±0.13**	229.7±13.7
Saffron Extract (0.2 g)	1.59±0.11*	69.1±8.1
Sildenafil (100 µM)	3.66±0.19**	289.3±7.5
Papaverine (100 µM)	1.05±0.1	11.7±6.1

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

Table 5: Effect of the hexane fractions of *A. marina* and saffron 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	—
Avicennia Extract (0.5g)	0.96±0.12	↓ 14.2±7.9
Saffron Extract (0.5g)	1.15±0.1	↑ 2.7±3.1
Sildenafil (50mg)	1.06±0.1	↓ 5.3±8.1

Pressure buildup started at 12 min. following the extract administration and reached to maximum after 2 min.

Table 6: Effect of *A. marina* and hexane extracts, sildenafil and papaverine on the rat intracavernosal pressure

Treatment (Dose) /kg	Route of Administration	Increase in the Intracavernosal pressure(mm/Hg)
Avicennia Extract (0.5g)	i.p.	4.8±0.3*
Saffron Extract (0.5g)	i.p.	1.4±0.8
Papaverine Hydrochloride (0.2g)	i.p.	3.3±0.5*
Sildenafil (2mg)	Intracavernosally	4.2±0.9*

**P*<0.05, N=4 compared with the basal level before administration of the drugs.

DISCUSSION

The results of this study clearly demonstrated the ability of the chloroformic/hexane extract of *A. marina* and the alcoholic/hexane extract of *C. sativus* to enhance the sexual activity of male rats in both behavioral 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. *A. marina* seemed to be a stronger sexual stimulant than both saffron and the standard drug sildenafil.

Previous studies revealed that both nitric oxide and non-nitric oxide induced elevation of cGMP are involved in correcting erectile dysfunction (Mcauley *et al.,* 2001). Thus, cyclic GMP was measured in this study. The biochemical studies that addressed the influence of the two herbs on the cyclic GMP within the corpora cavernosa and the release of testosterone revealed the ability of the two herbal extracts to increase the availability of cyclic GMP without any effect on testosterone release as there is no change in the plasma level of testosterone active metabolite-dihydrotestosterone. An increase in the intracellular cavernosal cyclic GMP content is usually linked with cavernosal smooth muscles relaxation, increase in penile blood flow and consequent erection (Porst *et al.,* 2003). A supplementary mechanism for the herbs-induced increase in the sexual activity may come from the antioxidant activity of the herbs constituents. Such an action may help to combat the accumulation of the known vasoconstrictors - the free oxygen radicals. Indeed, an antioxidant activity has been observed for flavone and flavonol constituents of *A. marina* (Vadlapudi and Naidu, 2009; Sun *et al.,* 2010) and also for its constituent α-tocopherol (Ramadan *et al.,* 2009). Similarly, saffron constituentscrocin and safranol are shown to be potent oxygen radicals scavengers (Assimopoulou *et al.,* 2005).

Furthermore, previous studies revealed an inherent vasodilatant and smooth muscle relaxant effects for saffron extracts and constituents as revealed by a decrease in the arterial blood pressure of the rat (Fatehi *et al.,* 2003) probably via a calcium channel blocking action (Abe *et al.,* 1999) mediated via safranal (Boskabady and Aslani, 2006) or crocin which is also shown to increase retinal blood flow (Xuan *et al.,* 1999).

In studies dealing with the sexual stimulant actions of drugs, various authors tend to measure the influence of the test drugs on the intracavernosal pressure. In studying drugs that are known to induce vascular actions, some researchers calculate the ratio between the induced increase in the intracavernosal pressure and the mean arterial pressure as an index of erectile function (Fiberg *et al.,* 1993), whereas others just report the effects of the examined drugs on the intracavernosal pressure in mmHg (Chion *et al.,* 1998). Our own experiments with the two herbs used in this study revealed that (i.p) injections of the extracts in the doses used in this study revealed minimal and non-significant cardiovascular changes. Thus, the extracts-induced increases in the intracavernosal

pressure were reported in mmHg just to provide further evidence to support the behavioral sexual outcomes observed.

In this study, no attempt was made to isolate and pin-point the actual candidates in the two herbs that may be involved in the observed behavioral, biochemical or functional sexual actions. *A. marina* is known to contain the triterpenoids lupeol, taraxerol and betulinic acid (Jia *et al.,* 2004; Feng *et al.,* 2007; Gaikwad *et al.,* 2010; Mahera *et al.,* 2011) and abietane diterpenoids such as 11-hydroxy-8,11,13-abietatriene 12-*O*-β-xylopyranoiside (Han *et al.,* 2008), together with marinoidal-glucosides such marinones A and B (Sun *et al.,* 2009) and iridoidal glucosides such as 10-*O*-[(E)-cinnamoyl]-geniposidic acid and 2'-*O*-[(E)-cinnamoyl] mussaenosidic acid (Shaker *et al.,* 2001; Sun *et al.,* 2008) and geniposide and mussaenoside (Koenig and Rimpler, 1985) and many others that included lyoniresinol 3α-*O*-β-D-gluco-pyranoside, avicennone D, avicenone E, salsaside A and avicennol C (Han *et al.,* 2008; Sun *et al.,* 2009) and the phenylpropanoid glycoside verbascoside (Fauvel *et al.,* 1993). One or more of these constituents acting alone or in concert can underlie the observed *A. marina*-induced sexual stimulant actions.

The situation is the same in case of saffron. No attempt was made to isolate the involved constituent. The stigmas of *C. sativus* are known to contain the carotenoid α-crocetin (or crocetin-1) and its glycoside crocin that gives the stigmas their golden yellow orange colour (Jose-Antonio, 2004; Champalab *et al.,* 2011) and picrocrocin with its aglycone safranal that gives the plant its aroma (Champalab *et al.,* 2011) together with the antioxidant carotenoids lycopene and zeaxanthin (Vijaya Bhargava, 2011).

Previous studies in rats showed an aphrodisiac action of the aqueous extracts of saffron and its constituent crocin (Hosseinzadeh *et al.,* 2008) but crocetin cannot be ruled out (Jose-Antonio, 2004).

On a broad basis, the results of this study point to the potential inherent sexual stimulant action of the chloroform/hexane fraction of *A. marina* and the alcohol/hexane fraction of *C. sativus* (saffron) in rats to an extent exceeding that of sildenafil in case of *A. marina*. It is hoped that further experimental and clinical studies may pave the way towards introduction of the herbs' active components as new competitors for the available phosphodiesterase 5 inhibitors such as sildenafil, tadalavil… etc. in the treatment of decreased libido, erectile dysfunction and impotence in males.

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