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Spilanthes acmella ethanolic flower extract: LC-MS alkylamide profiling and its effects on sexual behavior in male rats

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Abstract

According to Indian Systems of Medicine, *Spilanthes acmella* (L.) Murr. (Family-Asteraceae), is considered effective in the treatment of sexual deficiencies especially due to aging. In the present study, characterization of ethanolic extracts of the *Spilanthes acmella* flower and its effect on general mating pattern, penile erection and serum hormone levels of normal male Wistar albino rats were investigated and compared with sildenafil citrate. *In-vitro* nitric oxide release was also investigated in human corpus cavernosum cell line. As *N*-alkylamides are a promising group, their profiling was performed using a gradient reversed phase high performance liquid chromatography/electrospray ionization ion trap mass spectrometry (HPLC/ESI-MS) method on an embedded polar column. MS^1 and MS^2 fragmentation data were used for identification purposes. For assessment of sexual behavior, animals were divided into five groups of eight male rats. The extracts (50, 100 and 150 mg/kg body weight/day) and sildenafil citrate (5mg/kg body weight/day) (positive control) were administered orally for 28 days. The behavioral and sexual parameters were observed at day 0, 15, 28 and after a lapse of 7 and 14 days of discontinuance of drug treatment. Five *N*-isobutylamides, one 2-methylbutylamide and one 2-phenylethylamide were identified. The orally administered extract had a dose dependent positive effect on mounting frequency, intromission frequency and ejaculation frequency and the most significant effects ($p < 0.05$) were observed at 150 mg/kg treatment, even after a lapse of 7 and 14 days of discontinuance of drug treatment. A dose dependent effect was also observed on the FSH, LH and testosterone serum levels. With 150 mg/kg of ethanolic extract the values for FSH, LH and testosterone were 3.10±0.25 mlU/ml, 6.87±0.18 mlU/ml and 3.72±0.12

ng/ml respectively. *In-vitro* nitric oxide release was 21.7 ± 2.9 μM, which was significantly higher compared to the control group ($p < 0.01$). Sildenafil citrate exhibited also a significant effect on NO release, but no effect on hormone levels of rats was observed. The aphrodisiac potential of an ethanolic *Spilanthes acmella* extract was demonstrated *in-vitro and in-vivo*. *N*-Alkylamides might attribute to the improved sexual potential. Study lends support to the traditional utilization of *S. acmella* as a sexual stimulating agent.

Keywords: Aphrodisiac; Male sexual behavior; *N*-Alkylamides; Nitric oxide; *Spilanthes acmella*; Testosterone.

Introduction

Human search for sexual enhancers from natural substances is as old as civilization itself. Ancient history in most cultures helped society in its desire to improve the sexual experience as evident by writings in holy texts and sculptures in Hindu temples. For many years, people have searched for ways to achieve sexual desire, sexual health and sexual techniques (Jain et al., 2010).

Sexual function is an important component of quality of life and subjective well-being in humans. Sexual problems are widespread and adversely affect mood, and interpersonal functioning. The main problems are related to sexual desire and male erectile dysfunction. Successful treatment of sexual dysfunction may improve not only sexual relationships, but also the overall quality of life (Shin et al., 2010).

Sexual dysfunctions increase with ageing and etiological factors, including degenerative diseases, increase in injuries and stress associated with industrialized lifestyles. It can be treated by both medical and surgical modalities. However, plant-derived and herbal remedies continue to be a popular alternative (Rowland and Tai, 2003). For several hundred years, people around the world have used locally grown plants as supplements to energize, vitalize, and eventually to improve male sexual functions.

The availability of the large number of sex-improving drugs in the traditional Ayurvedic System of Medicine is a unique and distinctive feature of this system. In this system, Rasayana drugs constitute a special category, the uses of which are advocated for rejuvination, revitalisation and longevity. A special class of Rasayana drugs is known as Vrishya or Vajikarana. They are associated with an improvement of male sexual potency and thereby ensure a *supraja*, or better progeny. Traditionally, the main aim of using

Vajikaran was to achieve successful copulation for healthy reproduction, along with an improvement in sexual pleasure as an additional benefit. Vajikaran drugs are specially recommended to people suffering from sexual insufficiency and people in advanced age losing interest in sexual act or failing in sexual performance (Sharma et al., 2010). Besides having many specific drugs for enhancing sexual functions, the most commonly used is akarkara. Different plants are being referred to as akarkara, but the most prominent one is *Spilanthes acmella* (*S. acmella*). The akarkara plants are empirically used as powerfull aphrodisiac in traditional medicine practice in cases of sexual debility or depressed desire.

S. acmella has long been used in the traditional Indian systems of medicine for the treatment of various sexual inadequacies and is claimed to improve sexual functions in man. Other ethnopharmacological claims associated with *S. acmella* include its usage in treatment of rheumatism, inflammation, stimulant and as sialagogue for stammering, tongue paralysis, stomatitis, toothache, headache and treatment of asthma, rheumatism, fever, sore throat and hemorrhoids and gum infections (Prachayasittikul et al., 2009 and Vijeyaanandhi et al., 2007). In addition, its extract is traditionally added to nutritional supplement and cosmetics to accelerate repair of wrinkles which are caused due to vasoconstriction of skin. Pharmacologically, *S. acmella* is a potential vasodilator, antioxidant (Wongsawatkul et al., 2008) and immunomodulator (Savadi et al., 2010). Previous studies have demonstrated its diuretic, antibacterial, and anti-inflammatory activities (Ratnasooriya et al., 2004). Recently, it has also been demonstrated that spilanthol in *S. acmella* extracts permeates the skin and buccal mucosa (Boonen et al., 2010a, b).

Phytochemical analysis of ethanolic extract of *S. acmella* revealed that it is rich in *N*-alkylamide. The main *N*-alkylamide of *Lepidium meyenii* (Maca), known as macamide, and of *Anacyclus pyrethrum*, known as pellitorine, have been found effective in improvement of sexual behaviour (Cicero et al., 2001 and Sharma et al., 2010). Keeping in view the growing popularity and market interest in herbs for sexual problems, and lack of scientific studies on *S. acmella*, present investigation was undertaken to evaluate the scientific foundation for the concept of Vajikaran Rasayana.

Materials and methods

Animal stock

The protocol for experimentation was approved by Institutional Animal Ethics Committee of Dr. Hari Singh Gour University, Sagar, India (Animal Eths Comm/IE/98/Reg No379/01/ab/CPCSEA) and was in accordance with international standard on the care and use of experimental animals. Inbred, 40 sexually active Wistar strain male albino rats, weighing 150-180 g. Female from the same strain rats, used as stimulus for evaluation of sexual behavior, were prepared for experimentation, using the method reported by Agmo (Agmo, 2003). In brief, before all testing sessions, female estrus was induced by administration of estradiol benzoate (25 μg/rat), followed by progesterone (250 μg/rat), 48h later. Females were used between 4 and 8 h after the progesterone administration. Both steroids were purchased from Sigma (St.Louis, MO, USA). They were dissolved in arachis oil (Kriti, India) and injected subcutaneously in a volume of 0.1 ml/rat. The rats were housed at room temperature (24 ± 2°C) on a reversed day-night cycle (dark from 06:00 to 18:00) and relative humidity of 50–55%. They were fed with a standard pellet diet and water *ad libitum.*

Preparation of extracts

The plant *S. acmella* was identified and authenticated at Department of Botany, Dr. H. S. Gour University Sagar (M.P.) India and a herbarium has been deposited there as well (Herbarium No. Bot/02-8/2008). The flowers of the plant were collected from the area in vicinity of the campus and were dried at room temperature (25-35°C). Next, the flowers were reduced to powder and passed through a sieve (60 mesh), fed in a soxhlet extractor and extracted with ethanol (95%) till complete exhaustion. The extract was collected and dried under vacuum by using a rota vapor (Heidolph, Germany). The yield of the ethanolic extract was found to be 6.1% w/w. Before oral administration, the *S. acmella* extract was suspended in 1% sodium CMC.

HPLC/ESI-MS analytical *N*-alkylamide profiling

Analytical samples were prepared by dissolving the extract and reference material in a acetonitrile:water mixture (50/50, V/V), followed by filtration over a 0.45 µm nylon HPLC filter (Whatman, Dassel, Germany). The LC/MS apparatus consisted of a Spectra System SN4000 interface, SCM1000 degasser, P1000XR pump, AS3000 autosampler and was equipped with a Finnigan LCQ Classic ion trap mass spectrometer in positive ion mode (all Thermo, San José, CA, USA). Data were acquired using Xcalibur 2.0 software (Thermo, SanJosé, CA, USA). A Prevail RP C_{18} column (250 × 4.6 mm, 5 µm) with suitable guard cartridge (4.6 x 7.5 mm, 5µm) (both from Grace, Lokeren, Belgium) was used (Boonen et al., 2010b). The injection volume was 25 µl. The flow rate was set to 1.0 mL/min and the linear gradient used was as follows (where A = 1% acetic acid in ultrapure water and B = HPLC grade acetonitrile): t=0 min, A:B (80:20, V/V); t=0-150 min, A:B (10:90, V/V); t=150-151 min, A:B (80:20, V/V); t=151-166 min, A:B (80:20,

V/V). ESI was conducted using a capillary voltage of 3 kV. Nitrogen was used as the sheath and auxiliary gas with the heated capillary set at 275°C. MS-MS spectra were obtained by collision induced dissociation (CID) of the parent m/z, with the relative collision energy set at 35%. Identification was based on the m/z values and fragmentation ions, while quantification was performed using a laboratory reference material ("A. Vogel *Spilanthes*", Biohorma, Lummen, Belgium) with spilanthol (0.11% w/w) as the reference biomarker.

Treatment

Male rats were divided into five equally spaced groups. Group I served as blank and received only vehicle, *i.e.* normal saline. Group II, III and IV were given an oral daily dose of 50, 100 and 150 mg/kg *S. acmella* extract, respectively. Group V rats were administered daily and orally with 5mg/kg sildenafil citrate (a generous gift of Sun Pharma, India). The course of the treatment was 28 days.

Toxicity study

Two groups, containing six male and six female rats were used. The test group received an oral dose of 2 g/kg *S. acmella* extract. The rats in the control group received 1 ml of tap water. Behavioral parameters such as convulsions, sedation, and hyperactivity, grooming and accelerated breathing were observed. The animals were observed continuously for any behavioral changes or death for 1 h, then intermittently at 6 h, 24 h and finally 48 h after drug administration.

Functional studies

Penile erection

Penile erection was determined by using the method reported by Thakur et al. (2009). In brief, each male rat was placed in a transparent plexi glass cabin (60×40×40 cm) that was divided in half by 2 sheets of plastic fiber mesh, preventing contact but allowing auditory, visual, and olfactory stimuli. Ventral as well as lateral viewing and recording of the whole experimentation was facilitated by appropriately placing a mirror. After a 5-minute adaptation period, the test was started by placing an estrus female on the other side of the cage. Cages were cleaned before shifting the animals of different groups. The number of erections were recorded and tabulated. Erection in rats was marked by the visibility of the penis out of its sheath or by grooming of the penis, which is another indicator of penile erection in rats. Penile erection index (PEI) was calculated as per the methodology reported by Islam et al. (1991), *i.e.* by multiplying the percentage of rats exhibiting at least one episode of penile erection during 30-minute observation period with the mean number of penile erections: PEI= percentage of rats exhibiting penile erection × mean number of erections.

Parameters for sexual behavior analysis

The experimentation began by switching off the light at 08:00 A.M. After an interval of 20 minutes past turning off the light, the experimentation room was lit with a dim red illumination. The male rat was placed in a rectangular plexiglass chamber. After about 10 minutes, when the rat was acclimatized to the chamber condition, a sexually receptive female rat was dropped silently from one side of the chamber as stimulus. The observations in the 30 minute for sexual behavior were recorded for the following parameters (Thakur et al., 2009; Chauhan et al., 2009).

Mounting behavior Mount frequency (MF) was determined by counting the number of pre-ejaculatory mounts with and without intromission in given period of observation. Mount latency (ML) was calculated as the time lapse from the introduction of female to the occurrence of first mount.

Intromission behavior Intromission latency (IL) was calculated as the time when first intromission was observed after introduction of female in the cage. Intromission frequency (IF) was considered as total number of (pre-ejaculatory) intromission within 30 minutes.

Ejaculation behavior Ejaculation frequency (EF) was calculated as the number of ejaculations observed in the 30 minute period and Post-ejaculatory Interval (PEjI) was considered as the time interval between ejaculation and the first mount of the 30 minutes period.

Effects of drug after withdrawal of treatment

Daily dosing of the rats was stopped after 28 days of the treatment. In continuation, the observations of different parameters connoting sexual behavior were made on the 7th and 15th day after withdrawal of the treatment of respective groups (Sharma et al., 2010).

Serum FSH, LH and total testosterone hormone measurements

On the 28th day, blood was collected to measure serum FSH, LH and testosterone levels. Blood samples were spun at 2500 *g* for 10 minutes in a table top centrifuge. The serum samples obtained were analyzed to determine the concentration of FSH, LH and testosterone. Serum FSH and LH was measured (Erba Fertikit, Germany), according to the protocol provided with each kit. Serum concentration of total testosterone was measured by using a double antibody ELISA kit (Eiagen Testosterone kit, Italy). The

protocol used for the testosterone determination was according to the method described for the kit. Serum concentrations of hormones were determined in triplicate samples (Chauhan and Dixit, 2010).

In-vitro nitric oxide activity

DS-1 cells (human corpus cavernosum cell line) were routinely cultured in a humidified 5% CO2-95% air incubator in Dulbecco's modified eagles medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 20 μM L-glutamine, 1 mM pyruvate, 200 U/mL penicillin G, 200 μg/mL streptomycin and 4.5 mg/mL of D-glucose at 37°C. Cells were allowed to attach to the bottom of a 96-well plate overnight and were then exposed to the extract (10 mg/mL PBS) or placebo PBS (control) for 24 hours. The cell media was assayed for the concentrations of nitrate (NO_3^-) and nitrite (NO_2^-), the final *in-vivo* products of NO, using a commercial nitrate reductase preparation and Griess reagent kit (Cayman Chemical, USA) with the resultant absorbance being read at 550nm using a VictorX™ plate reader (Perkin Elmer USA) (Thakur et al., 2010).

Statistical analysis

Results are expressed as mean ± standard error mean (SEM). The groups were compared by ANOVA, followed by Dunnet's test. All the statistical analysis was carried out using Instat version, 2.1 software. Etholog 2.1 was used for computation of the behavioral parameters.

Results

LC-MS analytical *N*-alkylamide profiling

The identification of the *N*-alkylamides in the *S. acmella* extract was performed using the experimentally obtained m/z and their fragmentation pattern (Boonen et al., 2010b). LC-

MS revealed seven *N*-alkylamides in the ethanolic extract: five *N*-isobutylamides (IBA), one 2-methylbutylamide (MBA) and one 2-phenylethylamide (PEA). The total ion MS chromatogram is presented in Fig. 1. The most abundant *N*-alkylamide in the investigated *S. acmella* extract was (2*E*,4*E*,8*Z*,10*Z*)-*N*-isobutyl-dodeca-2,4,8,10-tetraenamide (0.71% w/w), while spilanthol was present at 0.20% w/w. Table 1 depicts the retention time of each of the present *N*-alkylamides, their precursor ion which corresponds to the molecular mass +1 ($M+H^+/Z$ with Z=1) and characteristic fragmentation information (formed by CID), as well as their proposed structure.

Toxicology

No acute toxicity of ethanolic extract was demonstrated; no mortality or changes in behaviour were observed in the control group or in the group of male and female rats treated with the *S. acmella* extract.

Penile erection

The administration of the extract resulted in a significant dose-dependent increase in PEI as compared to control group (Table 2). The PEI was nearly three times higher in the ethanolic extract dosed 150 mg/kg body weight (EE 150) and four times higher in the sildenafil citrate treated group during the treatment period.

Sexual behavior

The observations of the sexual behavior study are illustrated in Fig. 2,3,4,5,6, and 7 while the numerical data are given in Table A1. Treatment of the animals with the ethanolic *S. acmella* exctract brought a remarkable change in the sexual behavior. A decrease in ML, IL and PEI and increase in MF, IF and EF were observed, in a dose dependent manner. MF and EF were significantly altered in the treatment groups on the 15^{th} and 28^{th} day and

7 and 15 days after treatment withdrawal, whereas sildenafil citrate alters these parameters only on the 15^{th} and 28^{th} day of the treatment but not after withdrawal of the treatment.

Serum LH, FSH and testosterone levels

Compared to the control group, administration of 100 and 150 mg/kg body weight *S. acmella* extract for 28 days had significant effect on LH and FSH concentration in the serum (Table 3). In addition, serum testosterone level also increased significantly ($p<0.05$) in animals who received 50, 100 and 150 mg/kg *S. acmella* extract *in* comparison to the control group (Fig. 8). No effect of sildenafil citrate was observed on the hormone levels in rat sera.

***In-vitro* nitric oxide release**

Nitric oxide release was evaluated in DS-1 cell line and the relative % enhancement in nitric oxide release was compared to the solvent control (8.83 ± 0.98 μM). The *S. acmella* extract (10 mg/ml) exhibited a relative nitric oxide release of 21.7 ± 2.9 μM as compared to a nitric oxide release of 35.42 ± 2.7 μM with sildenafil citrate. The results are suggestive for the involvement of nitric oxide release mechanism for observed improvement in PEI by the *S. acmella* extract.

Discussion

Androgens have long been known to have a major stimulatory influence on several aspects of male sexual behavior, including penile erection. Sexual behavior is dependent on normal functioning of the hypothalamo-pituitary-gonadal axis. In most mammalian species studied, castration has been found to decrease substantially the erectile responses

to a variety of stimuli, whereas androgen replacement reversed these effects (Andersson, 2001).

Testosterone (T) is the main male gonadal hormone produced by the interstitial cells of the Leydig in the testis. T also helps in maintaining body shape, and increasing muscle mass and strength. The increase in testosterone should enhance androgen-dependent parameters such as mating behaviour and maintenance of spermatogenesis. FSH stimulates spermatogenesis in the Sertoli cells and LH (ICSH) stimulates synthesis and release of testosterone in the Leydig cells (Chauhan and Dixit, 2010). Testosterone may also facilitate male sexual behaviour by increasing dopamine release in the medial preoptic area and potentiating nitrergic neurotransmission (Hull et al., 1999; Putnam et al., 2001).

Present findings provide experimental evidence that the ethanolic extract of *S. acmella* flowers, used as a traditional medicine, possesses aphrodisiac properties. We demonstrated that the oral administration of its ethanolic extract was able to improve sexual performance, particularly of sexual arousal in male rats and that it promotes the expression of male sexual behaviour. Compared with the controls, the percentages of mounting and intromission in rats were significantly increased in animals by administration of the extract. Importantly, the extract has the capacity to produce long-term effects after even 14 days past cessation of treatment. The reduction of ML, IL and PEI is generally suggested to be indicative of an improved copulatory behaviour, particularly when observed together with an increase in mounting and ejaculating animals (Bitran and Hull, 1987).

The ability of different medicinal plants to improve sexual function as illustrated in the case of *Tribulus terrestris, Panax ginseng, Ferula hermonis* was ascribed to increased levels of testosterone in the serum (Zanoli et al., 2009). In another study, the effect of GnRH peptides substitution of Gly-NH2 with alkylamides maintained (methylamide and ethanolamide) or increased androgen activity up to 600% (propylamide and ethylamide) whereas substitution with larger amides (pyrolidineamide and morpholineamide) or D-amino acids decreased activity (Sealfon et al., 1997). It is possible that the alkylamide present in the extract may have an effect on androgen. There is a possibility of developing the ethanolic extract of *S. acmella* flowers as a therapeutic principle for stimulating male sexual activity, especially in cases where there are moderate sexual deficiencies. The presence of spilanthol and other alkylamides in the extract may be suspected as possible contributor to the observed effect of improved sexual function. The basis for such a premise is drawn from the libido enhancing properties of alkylamides isolated from the roots of *Lepidium sativum*. Oral administration of a purified lipidic extracts from *Lepidium meyenii* and *Anacyclus pyrethrum* increased intromissions and mounts frequency in normal mice (Zheng et al., 2000 and Sharma et al., 2010). In our study, ethanolic extract treated rats also showed increased intromissions and mount frequency. It is likely that alkylamide may mimic the action like testosterone or stimulate secretion of testosterone that improves sexual behaviour.

Enhanced erectile function is directly correlated to enhanced sexual performance in most cases (Fink et al., 2002). Penile erection is under direct control of NO release via neuronal, endothelial and inducible nitric oxide synthase activity (Burnett et al., 1992). The enhanced PI activity *in vivo* of the extracts is directly correlated with the inducible

NO (*i*NO) activity *in vitro* by the extracts of the drug, and is suggestive of improved erectile response and enhanced sexual pleasure, which are desirable attributes of any aphrodisiac agent. The present study also provides a good correlation between the *in vivo* improvement of penile erection and NO releasing activity of the extracts.

There was no toxic effect observed in the rats administered with these dosages of *S. acmella* flower extract in the present study. In fact, no effects were seen even at higher dosages as well. The exact molecular action mechanisms of the different *N*-alkylamides are not yet fully elucidated. It appears that the tingling effect of *e.g.* α-sanshool may be mediated by different ion-channel receptors on different types of sensory neurons, like the capsaicin (transient receptor potential vanilloid type 1 (TRPV1)), TRPA1, TRPM8 receptor, while more recently, emphasis is placed on distinct receptors like KCNK3, KCNK 9 and KCNK 18 (two-pore potassium channels) (Bautista et al., 2008). However, there is a consensus that several mechanisms are involved, with the relative importance dependent on the *N*-alkylamide structure (Viana, 2011).

Further investigations are in progress to ascertain the mechanism by which chemical constituents in *S. acmella* flowers ethanolic extract exerts potent aphrodisiac activity in males. The effect of the drug on female sexual behaviour and male fertility remains to be studied.

In conclusion, the present study provides evidence that the ethanolic extract of *S. acmella* flowers is a potent stimulator of sexual behaviour in male rats. There was an overall increase in the sexual behaviour parameters for the treated groups, as reflected in the increasement of MF, IF and EF, and reduction in ML, IL and PEI. These results were statistically significant even after discontinuing the treatment. The effect persisted,

suggesting encapacitation of the body for improved sexual activity. It is interesting to note that sildenafil citrate improved the sexual parameters only during the treatment and was ineffective after its withdrawal. Based on our observations, *S. acmella* flowers ethanolic extract can be considered to possess aphrodisiac properties.

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Conflict of interest

No conflict to disclose

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Figure legends

Fig.1. Detailed TIC-MS chromatogram of the identified *N*-alkylamides in the ethanolic *S.acmella* extract. **[**1: (2E,4Z)-N-isobutyl-2,4-undecadiene-8,10-diynamide, 2: (2Z)-N-phenethyl-2-nonene-6,8-diynamide, 3: (2E,6Z,8E)-N-isobutyl-2,6,8-decatrienamide, 4: (2E,7Z)-N-isobutyl-2,7-tridecadiene-10,12-diynamide, 5: (2E,7Z)-N-isobutyl-2,7-decadienamide, 6: (2E,6Z,8E)-N-(2-methylbutyl)-2,6,8-decatrienamide, 7: (2E,4E,8Z,10Z)-N-isobutyl-dodeca-2,4,8,10-tetraenamide**]**

Fig.2. Effects of ethanolic extracts of *S. acmella* on mount latency in male rats. All values are expressed as mean ± S.E.M, n=8; P*<0.05 and P**<0.01 Considered significant as compared to control.

Fig.3. Effects of ethanolic extracts of *S. acmella* on mount frequency in male rats. All values are expressed as mean ± S.E.M, n=8; P*<0.05 and P**<0.01 Considered significant as compared to control.

Fig.4. Effects of ethanolic extracts of *S. acmella* on intromission latency in male rats. All values are expressed as mean ± S.E.M, n=8; P*<0.05 and P**<0.01 Considered significant as compared to control.

Fig.5. Effects of ethanolic extracts of *S. acmella* on intromission frequency in male rats. All values are expressed as mean ± S.E.M, n=8; P*<0.05 and P**<0.01 Considered significant as compared to control.

Fig.6. Effects of ethanolic extracts of *S. acmella* on ejaculation frequency in male rats. All values are expressed as mean ± S.E.M, n=8; P*<0.05 and P**<0.01 Considered significant as compared to control.

Fig.7. Effects of ethanolic extracts of *S. acmella* on post-ejaculation latency in male rats. All values are expressed as mean ± S.E.M, n=8; P*<0.05 and P**<0.01 Considered significant as compared to control.

Fig.8. Effects of ethanolic extracts of *S. acmella* on serum FSH, LH and Testosterone level in male rats. All values are expressed as mean ± S.E.M, n=8; P*<0.05 and P**<0.01 Considered significant as compared to control.

Table legends

Table 1: Detected *N*-alkylamides in the ethanolic *S. acmella* extract.

Table 2: Effects of ethanolic extracts of S. acmella on penile erection index (PEI) in male rats.

Table 3: Effects of ethanolic extracts of *S. acmella* on FSH, LH and testosterone in male rats.

Fig.1. Detailed TIC-MS chromatogram of the identified N-alkylami

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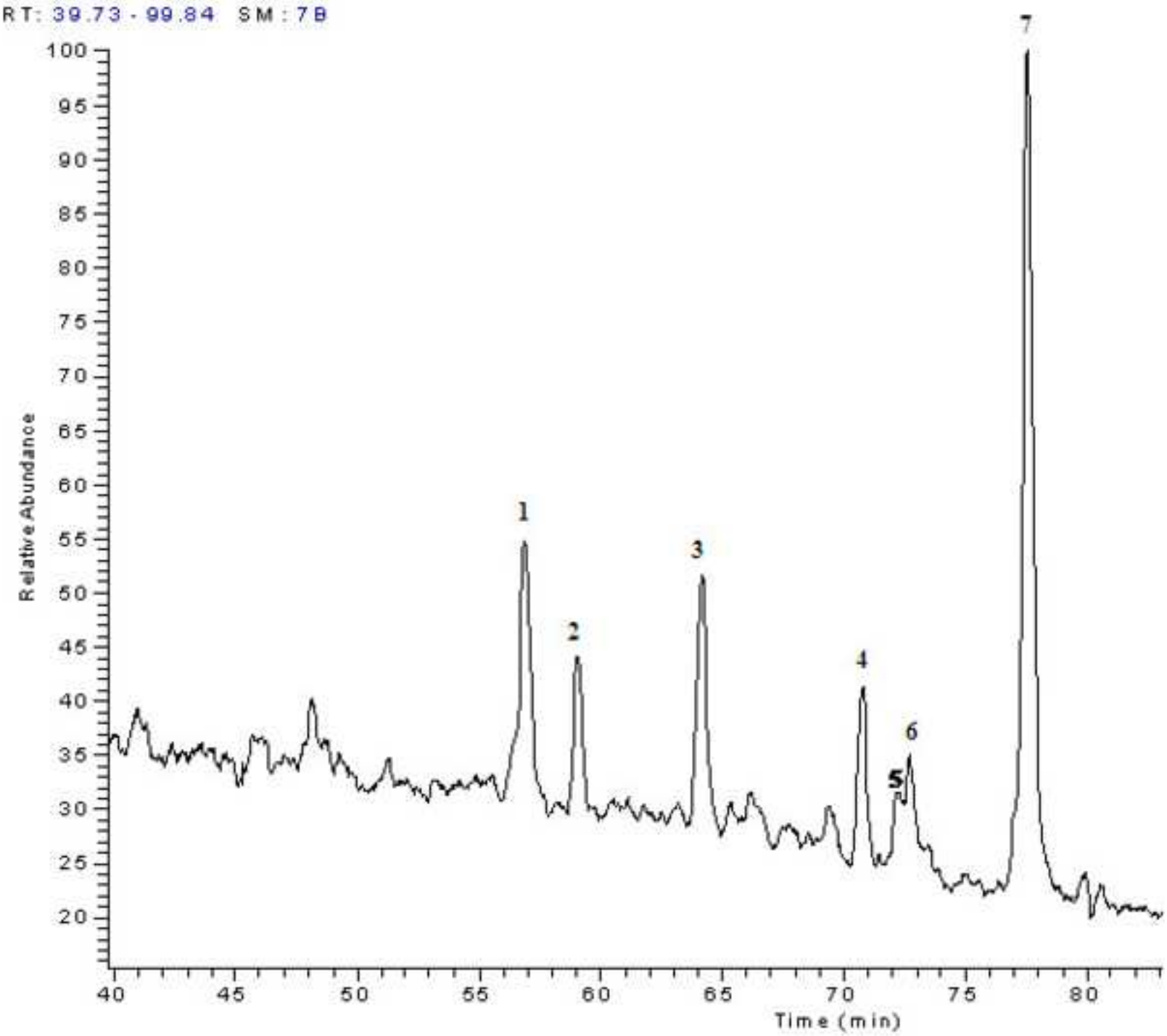

Fig.2. Effects of ethanolic extracts of S. acmella on mount late

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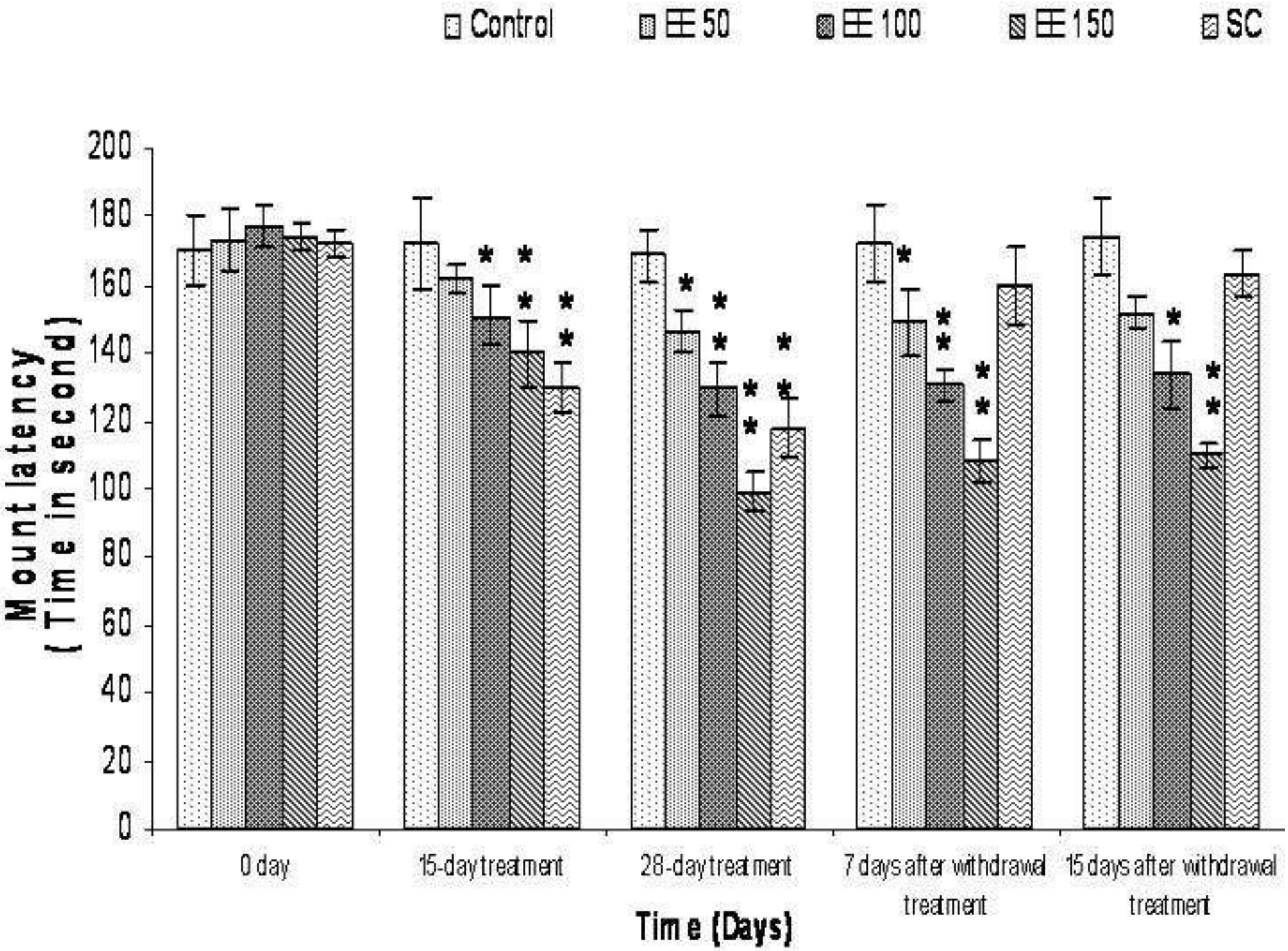

Fig.3. Effects of ethanolic extracts of S. acmella on mount freq

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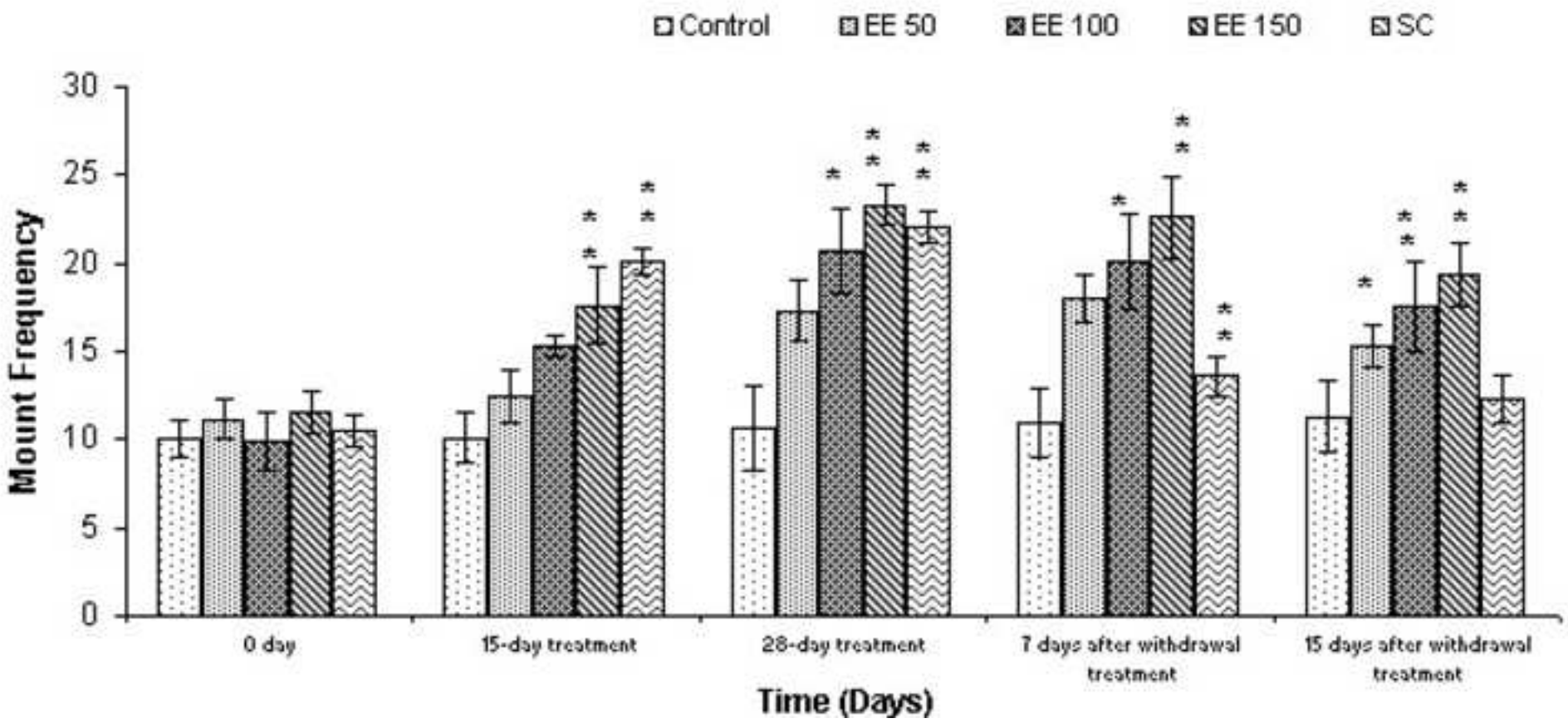

Fig.4. Effects of ethanolic extracts of S. acmella on intromissi

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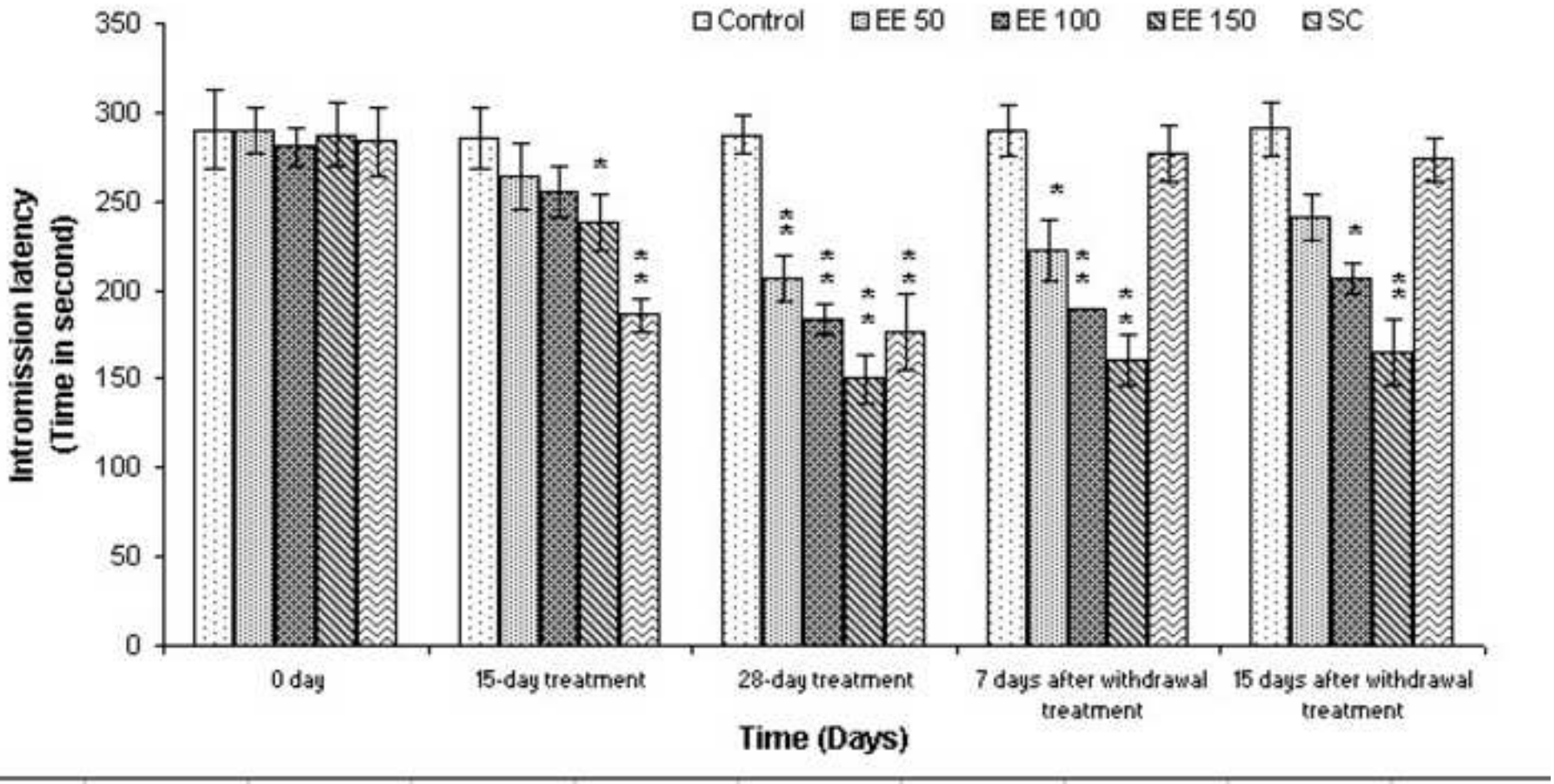

Fig.5. Effects of ethanolic extracts of S. acmella on intromissi

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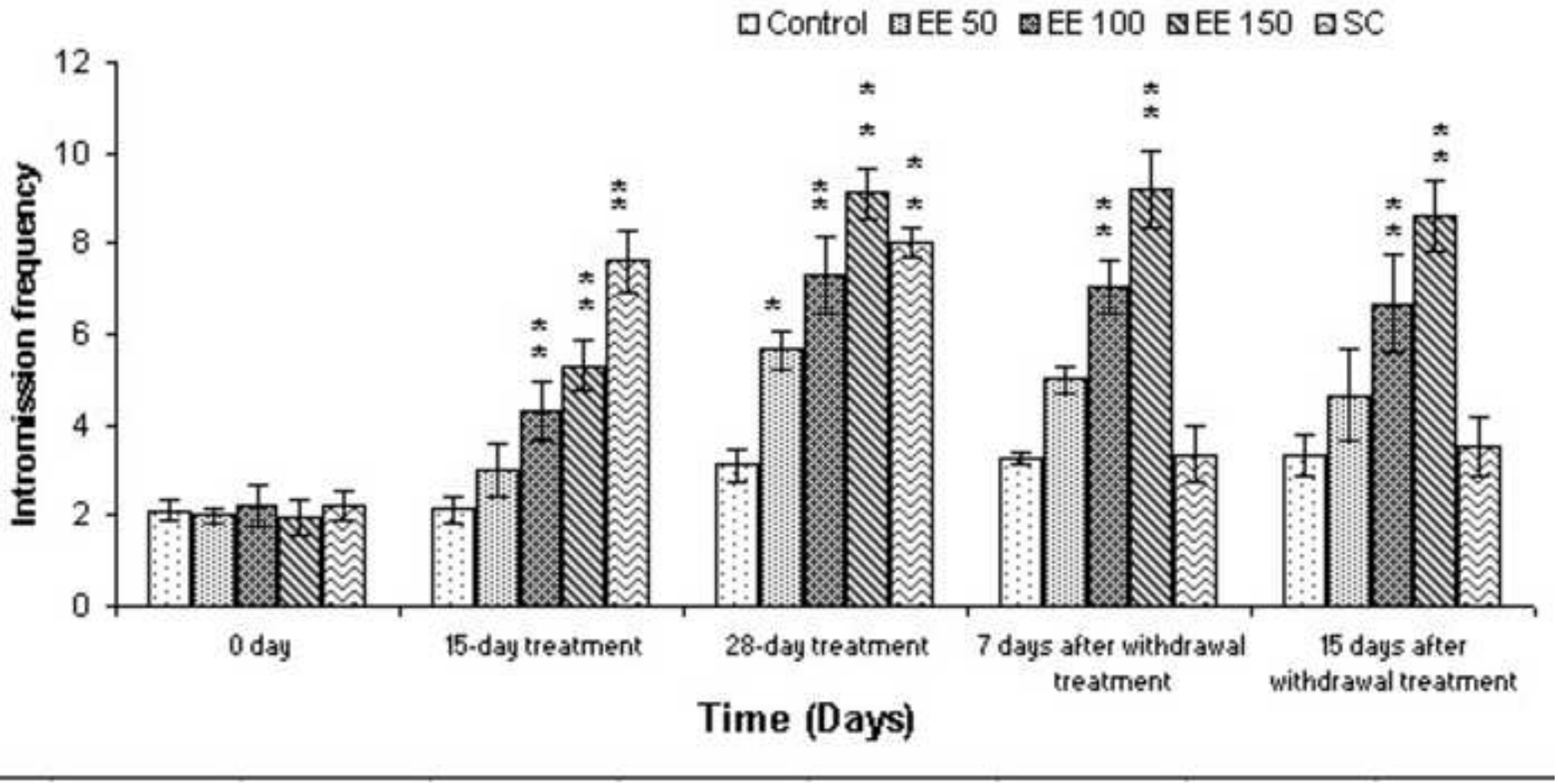

Fig.6. Effects of ethanolic extracts of S. acmella on ejaculatio

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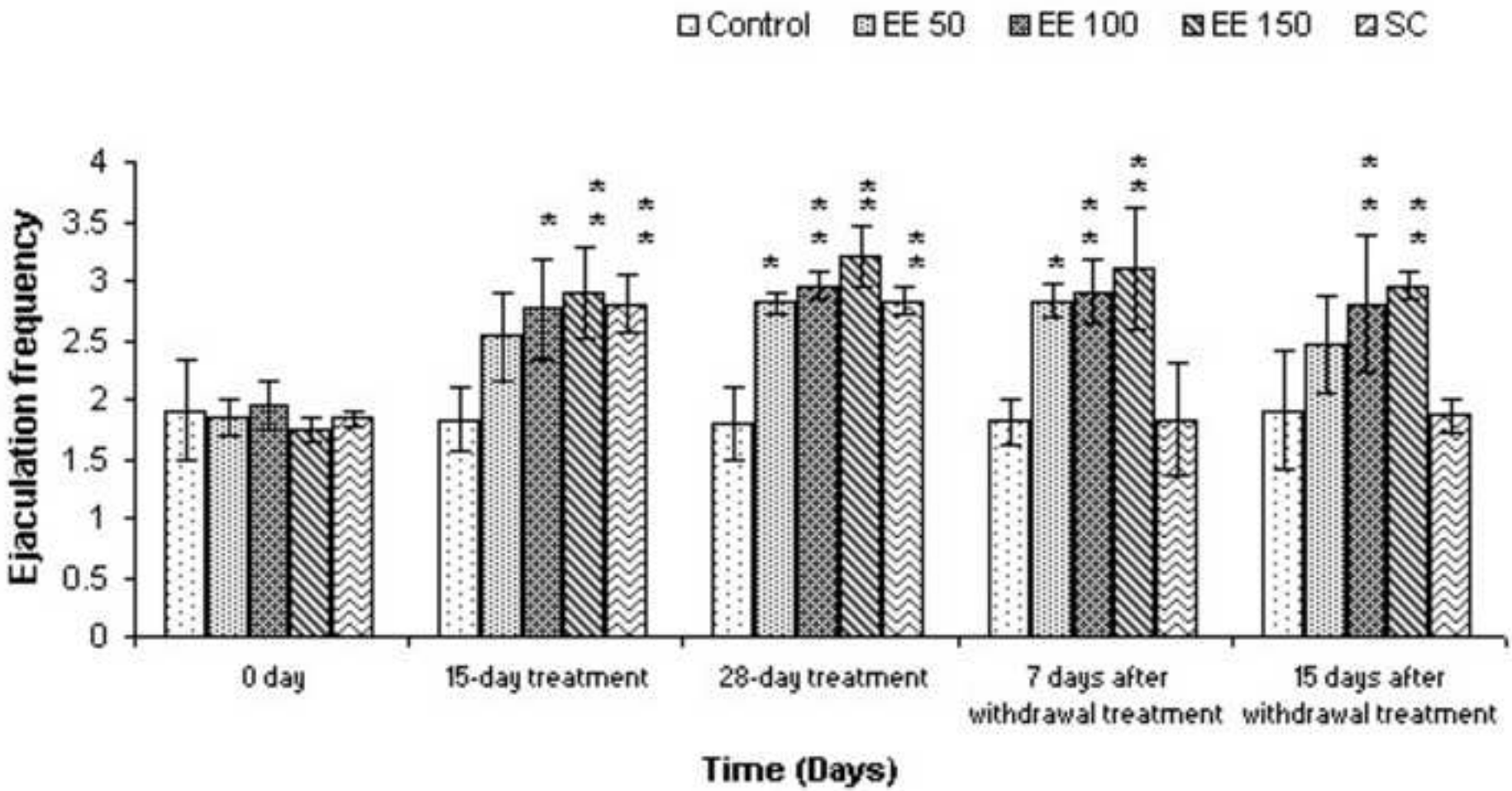

Fig.7. Effects of ethanolic extracts of S. acmella on post-ejacu

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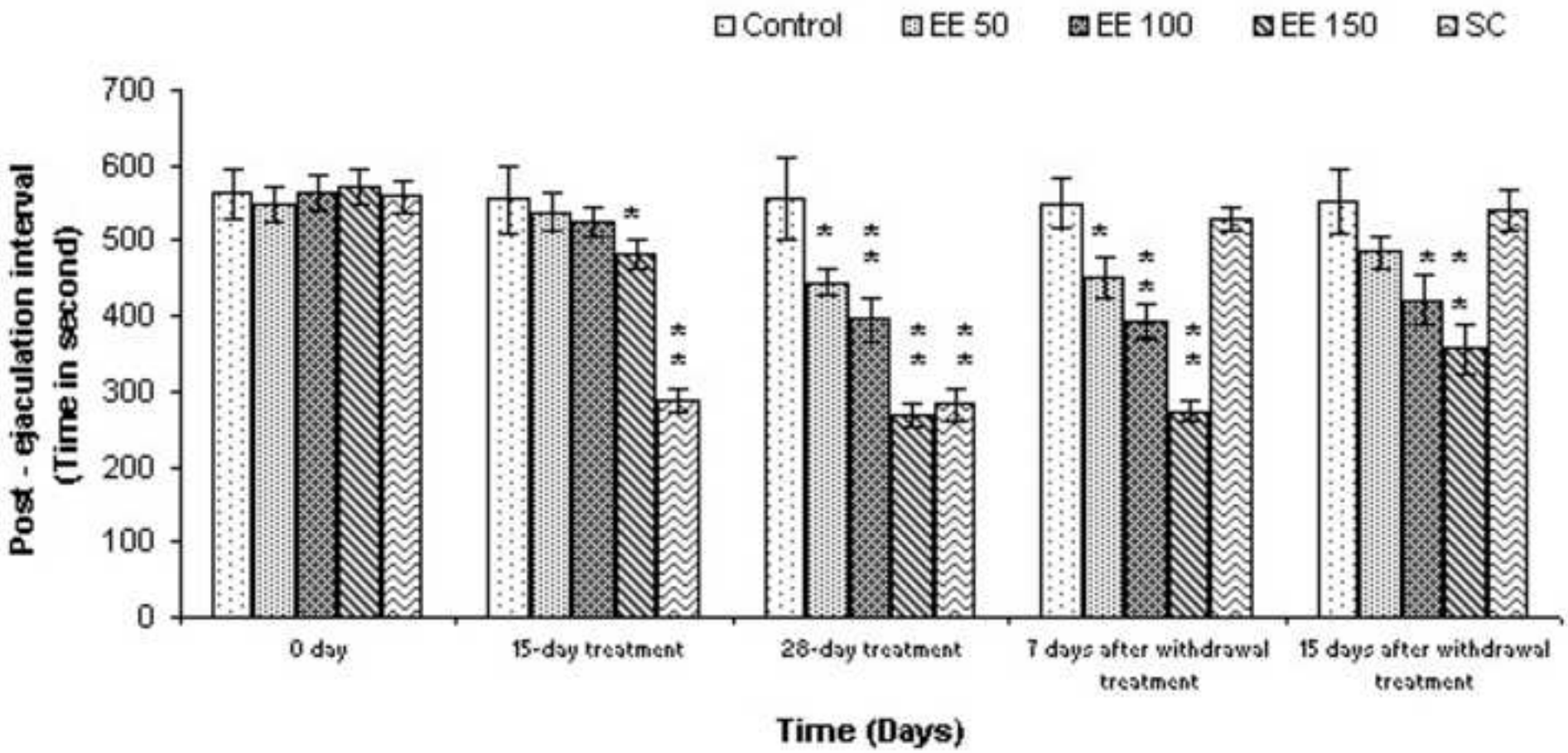

Fig.8. Effects of ethanolic extracts of S. acmella on serum FSH,
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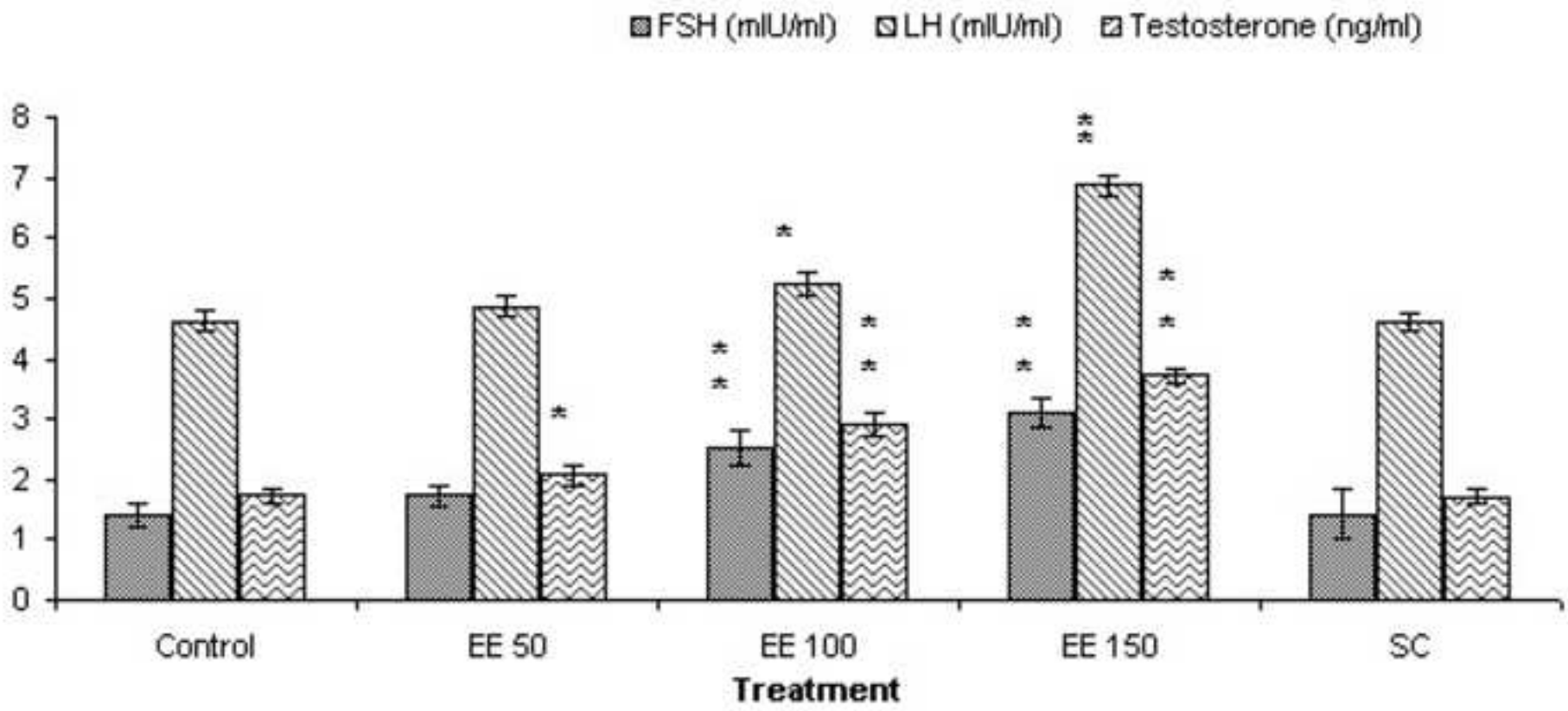


Table

Table 1: Detected *N*-alkylamides in the ethanolic *S. acmella* extract.

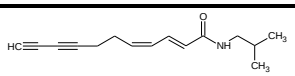
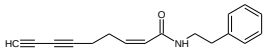
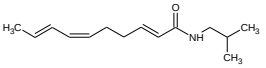
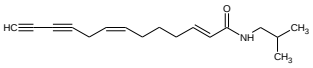
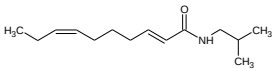
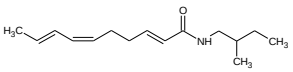
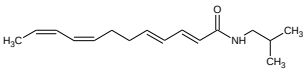
Peak no.	R_t (min)	Precursor ion	Fragment ions	Nomenclature	Structure	Content (% w/w)
1	56.9	230	**-101**; **-99**; **73**; **-56**	(2*E*,4*Z*)-*N*-isobutyl-2,4-undecadiene-8,10-diynamide		0.25
2	59.1	252	**-149**; **-147**; **-121**; **-104**	(2*Z*)-*N*-phenethyl-2-nonene-6,8-diynamide		0.11
3	64.2	222	**-115**; **-101**; **-99**; **-73**	(2*E*,6*Z*,8*E*)-*N*-isobutyl-2,6,8-decatrienamide		0.20
4	70.8	258	**-101;-99; -73**; **-56**	(2*E*,7*Z*)-*N*-isobutyl-2,7-tridecadiene-10,12-diynamide		0.12
5	72.2	224	**-101**; **-99**; **-73; -56**	(2*E*,7*Z*)-*N*-isobutyl-2,7-decadienamide		0.04
6	72.8	236	**-115**; **-113**; **-87**; **-70; -42**	(2*E*,6*Z*,8*E*)-*N*-(2-methylbutyl)-2,6,8-decatrienamide		0.07
7	77.6	248	**-99**; **-73; -56**	(2*E*,4*E*,8*Z*,10*Z*)-*N*-isobutyl-dodeca-2,4,8,10-tetraenamide		0.71

Table 2: Effects of ethanolic extracts of S. acmella on penile erection index (PEI) in male rats.

Group	Days of treatment			Days after withdrawal treatment	
	0	15	28	7	15
Control	23.9 ± 4.51	22.1 ± 2.40	23.9 ± 3.10	27.9 ± 3.39	27.6 ± 4.15
EE 50	21.2 ± 2.60	27.9± 2.61	38.81± 2.51	36.7± 4.40	30.4 ± 4.50
EE 100	21.1 ± 1.90	33.21 ± 7.01	63.8 ± 6.7**	61.4 ± 6.6**	47.15 ± 5.61*
EE 150	23.0 ± 2.20	44.4 ± 5.50**	69.5 ± 7.9**	69.5± 7.6**	63.8 ± 7.62**
SC	23.9 ± 1.10	80.5 ± 6.69**	94.1 ± 5.51**	27.56 ± 5.32	22.10 ± 2.90

* $p < 0.05$
** $p < 0.01$

Table 3: Effects of ethanolic extracts of S. acmella on FSH, LH and testosterone in male rats.

Group	FSH (mlU/ml)	LH (mlU/ml)	Testosterone (ng/ml)
Control	1.41±0.09	4.63±0.11	1.73±0.11
EE 50	1.73±0.18	4.87±0.10	2.07±0.07*
EE 100	2.52±0.27**	5.23±0.21*	2.91±0.09**
EE 150	3.10±0.25**	6.87±0.18**	3.72±0.12**
SC	1.42±0.41	4.60±0.15	1.71±0.13

* $p < 0.05$
** $p < 0.01$

Table AI: Effects of ethanolic extracts of *S. acmella* on sexual behavior in male rats.

Sexual behavior parameter	Groups	0 day	15-day treatment	28-day treatment	7 days after withdrawal treatment	15 days after withdrawal treatment
Mount latency (ML)	Control	170 ± 10.02	172 ± 9.15	168.6 ± 6.36	172.3 ± 4.33	174 ± 2.08
	EE 50	173 ± 13.15	162 ± 4.04	146.3 ± 8.39*	149.3 ± 9.81*	151.6± 7.26
	EE 100	177 ± 8.10	151 ± 5.77*	129.6 ± 7.93**	130.5 ± 5.88**	133.6 ± 8.8*
	EE 150	174 ± 11.14	140 ± 9.88**	99.3 ± 4.25**	108.6 ± 6.21**	110 ± 11.5**
	SC	172 ± 17.01	130 ± 4.61**	117.6 ± 9.71**	160.1 ± 3.32	163.3 ± 7.07
Mount frequency (MF)	Control	10.05 ± 1.05	10.12 ± 1.1	10.65 ± 1.7	11 ± 1.20	11.3 ± 0.88
	EE 50	11.14 ± 1.45	12.5 ± 1.51	17.3 ± 0.66	18 ± 2.15*	15.3± 0.76*
	EE 100	9.92 ± 2.42	15.3 ± 1.76	20.66 ± 2.40*	20.1± 1.15**	17.6 ± 0.88**
	EE 150	11.56 ± 1.95	17.6 ± 1.40**	23.3 ± 2.72**	22.6 ± 2.30**	19.3 ± 1.12**
	SC	10.54 ± 2.01	20.1 ± 1.15**	22.0 ± 2.57**	13.6 ± 1.80	12.3 ± 1.40
Intromission latency (IL)	Control	290.2 ± 22.46	285.6 ± 12.40	287.6 ± 10.91	289.3 ± 17.60	290.5± 19.23
	EE 50	289.6 ± 16.96	264.2 ± 19.06	206.3 ± 14.53**	221.7 ± 16.00**	241.3± 9.28
	EE 100	280.9 ± 11.40	255 ± 13.22	183.3 ± 8.9**	190.0 ± 13.5**	206.3± 21.80*
	EE 150	287.4 ± 14.54	238.3 ± 17.26*	150 ±12 .8**	160.6 ± 14.21**	165 ± 15.9**
	SC	283.6 ± 15.44	186.3 ± 13.17**	176.6 ± 8.71**	276.6 ± 18.19	273.3 ± 12.01
Intromission frequency (IF)	Control	2.11 ± 0.25	2.13 ± 0.18	3.1 ± 0.44	3.26 ± 0.37	3.33± 0.33
	EE 50	2.00 ± 0.29	3.0 ± 0.57	5.66 ± 0.63*	5.0 ± 0.55	4.66± 0.66
	EE 100	2.21 ± 0.33	4.3 ± 0.43**	7.31 ± 0.87**	7.04± 0.57**	6.66 ± 0.33**
	EE 150	1.96 ± 0.16	5.3 ± 0.31**	9.10 ± 0.57**	9.21 ± 0.84**	8.60 ± 0.62**
	SC	2.19 ± 0.46	7.60 ± 1.04**	8.01 ± 1.07**	3.33 ± 0.80	3.52± 0.66
Ejaculation frequency (EF)	Control	1.91 ± 0.42	1.83 ± 0.16	1.8 ± 0.20	1.81 ± 0.10	1.91± 0.06
	EE 50	1.85 ± 0.26	2.53 ± 0.37	2.81± 0.43*	2.83 ± 0.38*	2.46± 0.24
	EE 100	1.95 ± 0.31	2.76 ± 0.08*	2.96 ± 0.12**	2.90± 0.05**	2.80 ± 0.11**
	EE 150	1.75 ± 0.19	2.90 ± 0.15**	3.20 ± 0.27**	3.10 ± 0.57**	2.96 ± 0.88**
	SC	1.84 ± 0.51	2.80 ± 0.41**	2.83 ± 0.88**	1.83 ± 0.12	1.86± 0.13
Post-ejaculatory Interval (PEjI)	Control	562.42 ± 32.4	555.20 ± 22.4	556.00 ± 23.33	550.12 ± 23.09	552.60± 20.83
	EE 50	549.20 ± 43.9	538 ± 24.46	445.0 ± 18.93*	451.6 ± 19.65*	485.0± 14.43
	EE 100	565.24 ± 54.5	523.6 ± 17.49	395.3 ± 28.54**	393.33 ± 16.67**	421.6± 21.67*
	EE 150	571.44 ± 32.4	483.33 ± 26.6*	268.66 ± 22.5**	273.33 ± 14.53**	357.10± 14.57**
	SC	559.29 ± 41.4	288.3 ± 21.66**	282.0 ± 32.69**	528.2 ± 33.62	540.32 ± 28.86