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## Evaluation of Pharmacological Activities of Stay-On Power Capsules In Rats

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### ABSTRACT

The main objective of this study was to perform pharmacological evaluation of Stay-On Power Capsules in Rats namely the aphrodisiac activity. The present investigation deals with Pharmacological Studies (*Mating behaviour study, mating performance test, Hormonal analysis, Reproductive organ and spermal analysis*) of Stay-On Power Capsules in Rats administered orally by gavage to rats in accordance with the schedule Y of drugs and Cosmetic Act (2005). The acute toxicity study of Stay-On Power Capsules was done by up and down method. After the completion of acute toxicity studies, three different doses were selected for aphrodisiac activity such as 150, 300, and 600 mg/kg. Volume of oral administration was 1 ml/100 g of animals. Animals were randomly divided into five groups with six animals per group. Group I represented the control animals treated with normal water only; Groups II, III, and IV were treated with oral suspension of Stay-On Power Capsules at 18:00 h, at doses of 150, 300, and 600 mg/kg, respectively; and Group V served as a positive control treated with Sildenafil citrate of 5 mg/kg body weight. All the treatments were continued for 3 weeks only. Daily administration of Stay-On Power Capsules for 3 weeks to male rats resulted in a dose dependent increase in the mating performance as compared to the control group. The Stay-On Power Capsules at the dose of 150, 300, and 600 mg/kg showed 52.66%, 63.83%, 71% mating performance, respectively, against 38.33% of the control group, whereas the standard showed 79.66% mating performance. The Stay-On Power Capsules had significantly increasing (\*\*P < 0.01) effect on testosterone, LH, and FSH concentration in the serum in comparison to the control group. Oral administration of Stay-On Power Capsules for 3 weeks has been found to be relatively safe and effective in Mating behaviour study, mating performance test, Hormonal analysis, Reproductive organ and spermal analysis in rats.

**Keywords:** Mating behaviour study, mating performance test, Hormonal analysis, Reproductive organ and spermal analysis

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## INTRODUCTION

Herbal medicines are getting considerable interest worldwide. According to the report of the World Health Organization (WHO), herbal medicines are presently used for prevention and treatment of diseases by 80% of people in developing countries. In developed countries, the public interest in herbal prescriptions has also greatly increased (Aschwanden, 2001)<sup>1</sup>. Despite the wide use of medicinal plants, their safety and efficacy have not been fully investigated and more detailed analysis is therefore warranted for evaluation and standardization of herbal formulations (WHO, 2008)<sup>2</sup>.

Sexual dysfunction includes erectile dysfunction or impotence, ejaculation dysfunction, hypogonadism, etc. It is a serious public health problem among young as well as old men worldwide, with a prevalence of more than 20%. This dysfunction leads to risk for aging and other etiological factors, including degenerative diseases, increase in injuries, and stress associated with industrialized lifestyles<sup>3</sup>. Sexual relationship is among the most important social and biological relationship of human life by boosting the mood as well as interpersonal functioning. It does not only affect life expectancy, but also have a significant negative impact on an individual's wellbeing and quality of life<sup>4</sup>. People have searched for ways to achieve sexual desire or sexual techniques from ancient times<sup>5</sup>. In ancient history, most cultures helped society to improve the sexual life as evident by writings holy texts and sculptures in Hindu temples. Successful treatment of sexual dysfunctions may improve not only sexual relationships, but also the overall superiority of life<sup>6</sup>. It can be treated by both medical and surgical modalities. To achieve better sexual desire has led to the development and use of different substances known as aphrodisiacs. An aphrodisiac enhances sex drive or sexual pleasure by crossing the blood brain barrier and mimicking or stimulating some area of sexual arousal in the central nervous system<sup>7</sup>. These substances also act physiologically to increase blood flow to the penile area, or increase the duration of sexual activity by numbing the genital area or even mimic the burning sensation of sexual intercourse<sup>8,9</sup>.

Many indigenous plants have been claimed to have a sex stimulating effect in Ayurveda system. Among the several plants, *Terminalia catappa*<sup>10</sup>, *Allium tuberosum*<sup>11</sup>, *Bryonia laciniosa*<sup>12</sup>, *Elephant creeper*<sup>13</sup>, *Montana tomentosa*<sup>14</sup>, *Mucuna pruriens*<sup>15</sup>, *Cucurbita pepo*<sup>16</sup>, *Tribulus terrestris*<sup>17</sup>, *Hypericum perforatum*<sup>18</sup>, *Senecio cardiophyllus*<sup>19</sup>, *Ginkgo biloba*<sup>20</sup>, *Pausinystalia yohimbe*<sup>21</sup>, *Asteracantha longifolia*<sup>22</sup>, *Curculigo orchoides*, *Microdesmis keayana*<sup>23</sup>, etc., Most studies published on this regard have generally targeted one plant at a time even though in the traditional medicine, most of the plants are used in formulations of groups of two or four plants or even more. In the present study, Stay-On Power Capsules was studied on the scientific basis, as an

aphrodisiac combination and to determine the effects of the prepared formulation as suspension on sexual behaviour of male rats.

Stay On formulations of rare herbs include the highest quality Ginseng and Kesar which have proven benefits for rejuvenation. The ingredients of Stay-On Power Capsules consist of Ashwagandha, Ginseng, kesar, Safed Musli, Salam and Shilajeet. The present study is aimed to preclinically evaluate the Pharmacological activity of the Stay-On Power Capsules by administering it orally for 3 weeks.

## MATERIALS AND METHOD

### Materials

The test item was furnished from Shree Maruti Herbals 401, Kapurwala Bldg, Samuel Street, Nxt. to Bank of Baroda, Masjid Bandar (West), Mumbai, Maharashtra 400003 named as Stay-On Power Capsules. The physical appearances of the capsules were found to be brown powder in packets. Manufacture date 09/2016 and expiry date 08/2019. 3 bottles containing 30 capsules of Stay-On Power Capsules and two packets containing 25 gm of the powder were given by the supplier. The test drug was administered by dissolving in Normal water considered as vehicle for the test items. The standard was selected as Sildenafil Citrate of 5mg/kg body weight coded as SCRCHPS. The physical appearance of the standard was found to be White crystalline solid soluble in water, which was stored at 15-30 degree centigrade.

The reference item is any item used to provide a basis for comparison with the test item. In this experiment Sildenafil citrate of 5 mg/kg body weight is used. As it can be easily administered orally by making a solution of 5% in water and is well tolerated in rats.

### METHODS

#### Randomization, Numbering and Grouping of Animals

Both male and Female Wistar albino rats were procured from the animal house of Royal College of Pharmacy and Health Sciences with male rats weighing 191-262g and female rats weighing 164-201g or 8weeks aged. The animals were allowed to acclimatize 7 days prior to dosing. 6 animals were taken per group and maintained with standard laboratory diet and aqua guard water *ad libitum* in plastic bottles. The rats were housed 6 each of the same sex in polycarbonate cages provided with bedding of husk. The temperature was maintained in between 20 to 24 °C and relative humidity between 30 to 70%; 12 hours each of dark and light cycle was maintained. The doses administered were 150, 300, and 600 mg/kg body weight/animal per oral for male and 150, 300, and 600 mg/kg body weight/animal per oral for female.

#### For Aphrodisiac Activity

Sixty rats i.e. 30 males and 30 female healthy rats were divided into five groups of 5 rats per sex i.e. three test groups receiving the dose of 150, 300, and 600 mg/kg /animal/day and one control receiving normal water only. The last group shall serve as a positive control treated with Sildenafil Citrate of 5 mg/kg body weight. All the treatments are to be continued for 3 weeks only. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of dosing. Rats were assigned to six per cage sex wise and the individual animal was fur marked with picric acid. The females were nulliparous and not pregnant. The route of administration was selected as oral. The purpose of selecting Wistar albino rats for the study is that one of the rodent species is recommended as test system for the use in toxicity study as rodents are sensitive to toxins and drugs and hence widely used throughout industry for the evaluation of toxicity testing of orally administered drugs as historical data and evidence at the facility suggests.

### **Dose administration**

In Aphrodisiac Activity rats (n=6/sex/group) were administered vehicle (normal water), a dose of 150, 300, and 600 mg/kg body weight/ animal of Stay-On Power Capsules and Sildenafil Citrate of 5 mg/kg body weight daily up to three weeks by oral gavage.

### **Examination of major parameters prior to test**

All the animals selected for testing, were examined within 24 h before testing started by the same procedure to be used during the test examination. Animals have no abnormal hematology and serum chemistry parameters.

The present investigation deals with Pharmacological Studies (*Mating behaviour study, mating performance test, Hormonal analysis, Reproductive organ and spermal analysis*) of Stay-On Power Capsules in Rodents administered orally by gavage to rats in accordance with the schedule Y of drugs and Cosmetic Act (2005). The acute toxicity study of Stay-On Power Capsules was done by up and down method. After the completion of acute toxicity studies, three different doses were selected for aphrodisiac activity such as 150, 300, and 600 mg/kg. Volume of oral administration was 1 ml/100 g of animals. Animals were randomly divided into five groups with six animals per group. Group I represented the control animals treated with normal water only; Groups II, III, and IV were treated with oral suspension of Stay-On Power Capsules at 18:00 h, at doses of 150, 300, and 600 mg/kg, respectively; and Group V served as a positive control treated with Sildenafil citrate of 5 mg/kg body weight. All the treatments were continued for 3 weeks only.

### **Mating behaviour study**

Mating behaviour studies were carried out in a separate room under dim red illumination according to the standard procedure. Healthy male animals showing brisk sexual activity and female animals showing regular oestrus cycle were selected for the study. The male animals were placed in a rectangular Plexiglas chamber, 10 minutes before the introduction of a primed female and so as to get acclimatized to the chamber conditions. The primed female was then introduced into the chamber with one female to one male ratio and the mating behaviours were observed for first week and third week after commencement of the Stay-On Power Capsules treatment. The following mating behaviour parameters were recorded: (a) **Mount frequency (MF)**: The number of mounts without intromission from the time of introduction of the female until ejaculation; (b) **Intromission frequency (IF)**: The number of intromissions from the time of introduction of the female until ejaculation; (c) **Mount latency (ML)**: The time interval between the introduction of the female and the first mount by the male; (d) **Intromission latency (IL)**: The interval from the time of introduction of the female to the first intromission by the male (characterized by pelvic thrusting and springing dismount) ; (e) **Ejaculation latency (EL)**: The time interval between the first intromission and ejaculation (characterized by longer, deeper pelvic thrusting, and slow dismount followed by a period of inactivity), (f) **Post-ejaculatory interval (PEI)**: The time interval between ejaculation and the first intromission of the following series. The experiment was terminated when the male animal begins to mount the female followed by intromission after a brief period of inactivity (which normally results following ejaculation). The values of the observed parameters were measured at first week and third week of drug administration and compared with control<sup>24, 25</sup>.

### **Mating performance test**

After 3-week treatment, the male rat of each group was placed in separate cages with oestrus female animals for 1 day (male: female = 1:5). The next day morning, the vaginal smear of each female animal was examined under a microscope for the presence of sperm. The number of sperm positive females was recorded in each experimental group and compared with control<sup>25</sup>.

### **Hormonal analysis**

The blood was collected from retro orbital venous plexus of all animals after termination of experiment. Blood samples were spun at 2500 rpm for 10 min in a table top centrifuge. The serum samples were separated to measure the concentration of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. Serum FSH was measured by a radioimmunoassay kit (Board of Radiation and Isotope Technology, Mumbai, India) ; FSH concentration was estimated by a microplate chemiluminescence immunoassay (CLIA) kit; and total testosterone was measured

by a double antibody ELISA kit (Eiagen Testosterone kit, Italy), according to the protocol provided with each kit<sup>26</sup>.

### **Reproductive organ and sperm analysis**

At the end of study, the animals were killed by an overdose of anesthesia. Immediately after the respiration ceased, the animals were fixed by transcardial perfusion with normal saline after flushing the blood. Before perfusion, right hand side of the epididymis was removed and used for sperm analysis and left hand side was used for a morphological study. Main and accessory reproductive organs were dissected and weighed<sup>15</sup>.

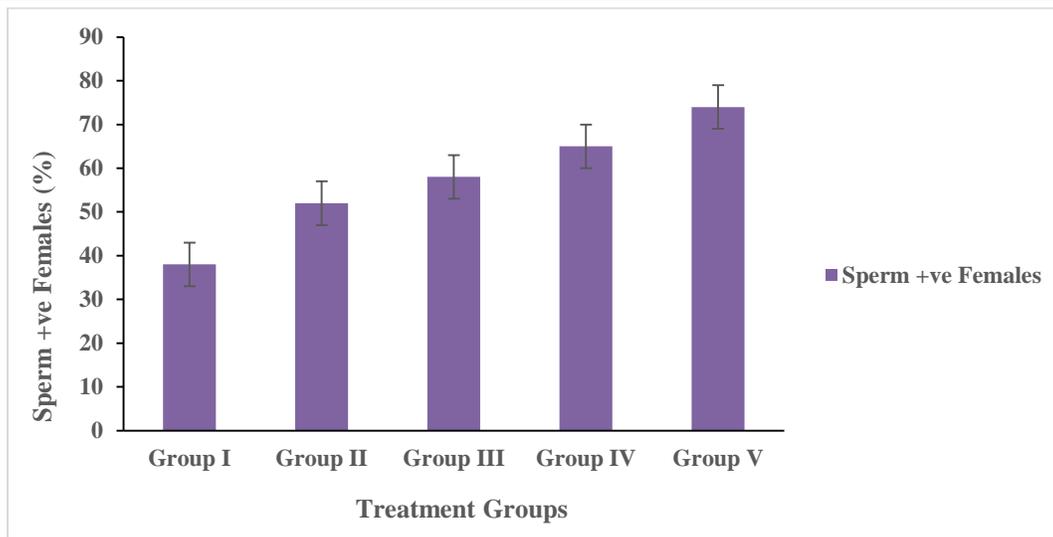
## **RESULTS AND DISCUSSION**

### **Effect of Stay-On Power Capsules on mating behaviour of rats**

A mating behaviour study revealed that continuous administration for 3 weeks, all the doses of Stay-On Power Capsules were able to significantly decrease mount and intromission latencies, when compared to vehicle control and standard drug treated rats. It also significantly decreases the ejaculatory latency and post ejaculatory interval. Ultimately, it resulted in an increased percentage of mounting frequency and intromission frequency in comparison to control and standard drug [Table 1]. However, the Stay-On Power Capsules at 600 mg/kg significantly increased the frequency of mounting and intromission and other reflexes of sexual behaviour, as nearly equal to standard drug. Also, the precoital sexual behaviours, such as chasing, nosing, and anogenital sniffing, were prominently observed in this group. All the doses of Stay-On Power Capsules were followed by dose dependent progression at first and third week interval consecutively on the mating behaviour of male rats as shown in Table 1. There was an overall increase in the sexual behaviour parameters in Stay-On Power Capsules treated groups of rats as reflected in MF, IF and EF, and reduction in ML, IL, EL, and PEI. These results were also statistically significant.

### **Effect of Stay-On Power Capsules on mating performance of rats**

In Figure IV, daily administration of Stay-On Power Capsules for 3 weeks to male rats resulted in a dose dependent increase in the mating performance as compared to the control group. The Stay-On Power Capsules as the dose of 150, 300, and 600 mg/kg showed 52.66%, 63.83%, 71% mating performance, respectively, against 38.33% of the control group, whereas the standard showed 79.66% mating performance. The Stay-On Power Capsules of 600 mg/kg showed closely resemblance with standard treatment and plays a significant role in mating performance of rats as compared to control.

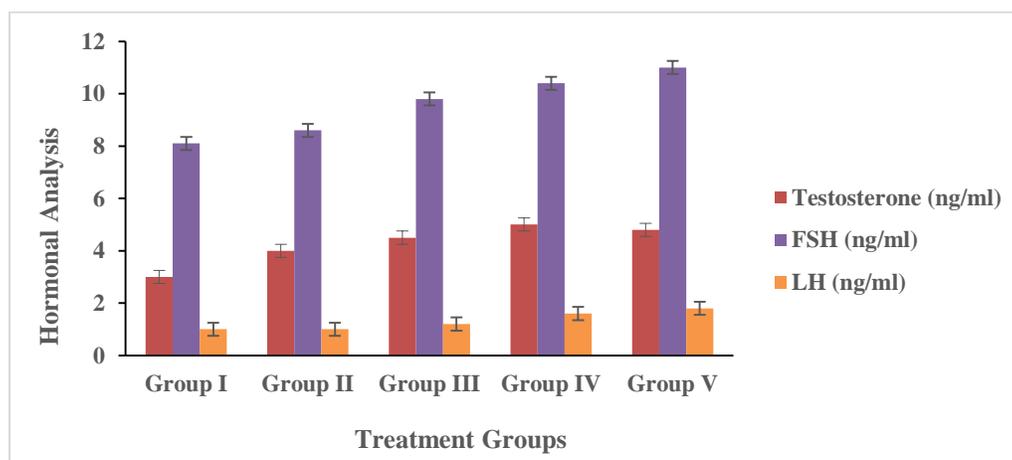


**Figure I: Effect of Stay-On Power Capsules on mating performance in male rats.**

All values were expressed as Mean ± SD (n=6); \*\*P < 0.01 considered significant as compared to control

**Effect of Stay-On Power Capsules on serum testosterone, LH and FSH in male rats**

The Stay-On Power Capsules had significant (\*\*P < 0.01) effect on testosterone, LH, and FSH concentration in the serum in comparison to the control group as shown in Figure 2. The level of testosterone, LH, and FSH increased gradually with dose dependency in all the experimental groups. The dose of Stay-On Power Capsules 600 mg/kg showed an increase of serum hormonal level as nearly as standard.



**Figure II: Effects of Stay-On Power Capsules on serum testosterone, FSH and LH level in male rats**

All values were expressed as Mean ± SD (n=6); \*\*P < 0.01 considered significant as compared to control

**Effect of Stay-On Power Capsules on testes body weight ratio and sperm count after termination**

The effect of the Stay-On Power Capsules on sexual organ and body weight is summarized in Table 2. After 3 week of treatment, the Stay-On Power Capsules showed increasing ratio of testes body weight in a dose dependent manner, and also found significance with control. The epididymal sperm parameters revealed an increase in the number of sperms in all tested groups as compared to control, i.e. 190, 220, 235, 264, and 287 million/ml in groups I, II, III, IV, and V, respectively.

**Table 1: Effect of Stay-On Power Capsules on testes-body weight ratio in male rats**

| <b>Groups</b> | <b>Testes-Body weight ratio</b> | <b>Sperm count (million/ml)</b> |
|---------------|---------------------------------|---------------------------------|
| Group I       | 0.007±0.001                     | 190±10.21                       |
| Group II      | 0.016±0.002**                   | 220±23.01*                      |
| Group III     | 0.014±0.001**                   | 235±17.23**                     |
| Group IV      | 0.013±0.003**                   | 264±13.71**                     |
| Group V       | 0.10±0.001*                     | 287±19.41**                     |

All values were expressed as Mean ± SD (n=6); \*\*P<0.05 considered significant as compared to control, Stay-On Power Capsules

**Table 2: Effect of Stay-On Power Capsules on mating behaviour after 1 week and 3-week treatment in male rats**

| MB  | Group I    |            | Group II     |               | Group III     |              | Group IV      |               | Group V      |             |
|-----|------------|------------|--------------|---------------|---------------|--------------|---------------|---------------|--------------|-------------|
|     | 1 week     | 3 week     | 1 week       | 3 week        | 1 week        | 3 week       | 1 week        | 3 week        | 1 week       | 3 week      |
| ML  | 9.89±0.19  | 10.63±0.87 | 7.61±1.13**  | 8.12±1.14**   | 5.84±0.78**   | 5.03±0.98**  | 4.32±0.65**   | 3.11±0.87**   | 2.03±0.05**  | 1.91±0.09** |
| IL  | 9.93±1.32  | 10.97±1.24 | 7.19±1.13**  | 8.53±1.83*    | 6.82±0.78**   | 5.63±1.35**  | 4.91±0.359**  | 4.01±1.46**   | 1.96±0.58**  | 1.66±1.69** |
| EL  | 236±0.89   | 249±1.96   | 209±2.21**   | 253±2.15*     | 249±1.52**    | 360±2.58**   | 373±2.14**    | 571±2.68**    | 1278±0.951** | 1296±2.41** |
| PEI | 443±3.21   | 453±2.15   | 448±3.56NS   | 461±2.68**    | 553±4.21**    | 627±3.24**   | 637±4.97**    | 697±1.98**    | 7.83±3.58**  | 4.72±2.67** |
| NI  | 5.8±0.59   | 5.1±0.88   | 5.9±0.76NS   | 6.1±0.96NS    | 6±0.68NS      | 6.3±0.54     | 6.1±0.32 NS   | 6.4±0.78*     | 6.97±0.92*   | 6.93±0.31** |
| III | 16.37±1.24 | 14.32±2.12 | 16.21±1.58NS | 16.09±2.52NS  | 15.14±2.04NS  | 14.48±2.36   | 14.31±1.68NS  | 12.28±1.97NS  | 7.21±1.08*   | 7.13±1.22** |
| NM  | 5.63±0.78  | 5.42±0.69  | 5.71±0.89NS  | 6.09±0.25NS   | 5.93±0.32NS   | 6.21±0.58    | 5.98±0.65NS   | 6.28±0.67NS   | 6.86±0.37*   | 7.03±0.57** |
| MF  | 70.48±0.78 | 68.23±7.27 | 72.48±0.88** | 75.63±6.86NS  | 75.49±0.39**  | 80.33±6.21** | 78.29±0.78**  | 85.63±5.98**  | 193±0.65**   | 207±6.03**  |
| IF  | 76.41±4.65 | 79.31±5.69 | 98.23±4.21** | 141.35±5.94** | 101.35±5.10** | 135.23±6.32  | 129.23±4.21** | 156.31±5.54** | 186±2.13**   | 209±5.09**  |

All values were expressed as Mean± SD (n=6), significant difference from control, \*P<0.01, NS=Not Significant, MB=Mating Behaviour  
ML=Mounting Latency, IL=Intromission Latency, EL=Ejaculation Latency, PEI=Post Ejaculation interval, NI=Number of Intromission, III-  
Inter-Intromission Interval, NM=Number of Mount, MF=Mounting Frequency, IF=Intromission Frequency

This study examined the effect of Stay-On Power Capsules on male sexual competence in rats, with sildenafil citrate as positive reference drug. To the best of our knowledge, this is the first study to report the prepared Stay-On Power Capsules enhanced the sexual behaviours of male rats compared with control. The present study provides special evidence that the Stay-On Power Capsules is a potent stimulator of sexual behaviour, particularly on sexual arousal in male rats.

The mating behaviour study revealed that the doses of prepared Stay-On Power Capsules significantly increased MF and IF, compared with the control group, though the effect was less than that of standard. All the doses of Stay-On Power Capsules also caused significant reductions in ML and IL, compared with control animals, while highly significant decreases in ML and IL were observed in animals treated with standard. MF and IF are considered to be indices of libido and potency, while ML and IL are also indicators of sexual arousal. The significant increases in MF and IF and the decreases in ML and IL indicate that libido and potency were enhanced by prepared Stay-On Power Capsules. Furthermore, the prolongation of EL is an indicator of prolonged duration of coitus. PEI is considered to be an index of potency, libido, and the rate of recovery from exhaustion after the first series of mating. This indicates that the treatment of different doses of Stay-On Power Capsules remarkably delayed EL, with no negative effect on the other parameters of sexual behaviour, and with no locomotor alterations throughout the observation period. The delayed EL and increased penile erection in treated male rats indicated the involvement of NO in the intervention. These observations support the role of Stay-On Power Capsules in improvising sexual function.

The continued administration of various doses of Stay-On Power Capsules for 3 weeks increased testosterone and LH levels. An increase in testosterone level has been associated with increase of sexual desire, penile tumescence, and rigidity, as well as the accessory muscles which help to provide additional sexual activity. Research with various animal and human models indicates there is a strong correlation between sexual behaviour and brain neurotransmitters like dopamine, 5-HT etc. The motor control of ejaculation in animals is modulated by serotonin and its receptors. Testosterone may also facilitate male sexual behaviour by increasing dopamine release in the medial preoptic area and potentiating nitrenergic neurotransmission in brain, which resulted in stimulation of hypothalamic-pituitary-gonadal axis. Also, increase in the testicular weight indicates the number as well as motility of sperms. Increased serum testosterone levels after administration of Stay-On Power Capsules could thus be considered as one of the contributing factors responsible for the overall increased sexual performance in the treated groups, especially for the lengthening of

EL and increased copulatory ability in rats. Overall, these results suggest that Stay-On Power Capsules at dose 600 mg/kg might represent an interesting alternative for the treatment of pretreatment ejaculation.

There are some possible bioactive agents responsible for increasing endogenous testosterone levels and enhancing male sexual behaviour. The mechanism of these agents includes steroids by rising androgen production flavonoids by enhancing testosterone synthesis or by preventing its metabolic degradation; alkaloids by dilating the blood vessels in the sexual organs; and saponins by activating gonadal tissues and CNS via NO-dependent mechanism. Thus, the improvements in sexual function demonstrated in the current study might be due to the presence of such compounds in prepared Stay-On Power Capsules.

Organ-body weight ratio is an index of inflammation or cellular constriction. The increase in the testes-body weight ratio observed [Table 2] may be attributed to increase the secretory activity of the testes, i.e. increase in the concentrations of testosterone, LH, FSH, cholesterol, protein, sialic acid, etc., Increased testicular weight and high protein concentration of the testes indicate enhancement of testicular growth as FSH. Testosterone, LH, and FSH are hormonal markers of androgenicity. Increase of testicular weight and hormonal concentration indicates the presence of androgenic potential in prepared Stay-On Power Capsules. FSH is responsible for the initiation, maintenance, and production of normal sperms in pubertal rats. The significant increase in the serum FSH suggests an enhancement of sperm cell in sertoli cells. Increase of sperm count was also observed of 3-week treatment of Stay-On Power Capsules.

The study concluded that the cumulative dose of Stay-On Power Capsules could enhance overall sexual function and performance in male rats by increasing the levels of FSH, LH, testosterone, spermatozoa concentration. The results suggest that the prepared Stay-On Power Capsules may be a new promising aphrodisiac combination, which can be used to improve the sex life of many troubled men. This aphrodisiac property may be due to possible synergistic action of selected plants used in the prepared Stay-On Power Capsules. However, further studies are warranted to establish molecular mechanism for aphrodisiac activity.

## CONCLUSION

Oral administration of Stay-On Power Capsules for 3 weeks has been found to be relatively safe and effective in Mating behaviour study, mating performance test, Hormonal analysis, Reproductive organ and spermal analysis in rats.

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