

**EVALUATION OF POTENTIAL APHRODISIAC ACTIVITY OF HENHANCE- A  
POLYHERBAL FORMULATION IN MALE ALBINO RATS**

**Ruchismita Ghosh<sup>1</sup>, Biplav Syngkli<sup>1</sup>, I. Aparna Lakshmi<sup>1</sup>, Dr. Shivalinge Gowda K. P.\*<sup>1</sup>, Nataraj Loganayaki<sup>2</sup>,  
Khader Shareef K. S.<sup>2</sup> and Venkateswarlu K.<sup>2</sup>**

<sup>1</sup>Dept of Pharmacology, PES College of Pharmacy.

<sup>2</sup>Suguna Foods Pvt Ltd, Suguna Lifeherbs, Herbal Division, 1057 Avinashi Road, 5th Floor, Jaya Enclave, Coimbatore,  
Tamil Nadu, India –641018.

**\*Corresponding Author: Prof. Dr. Shivalinge Gowda K. P.**

Head, Dept of Pharmacology, PES College of Pharmacy.

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**ABSTARCT**

**Aim:** The present study was aimed to evaluate aphrodisiac activity of HENhance-a polyherbal formulation developed by Suguna Foods Pvt Ltd in male albino rats. **Materials & Methods:** The acute toxicity of HENhance was evaluated by standard OECD guidelines up to a dose of 2000mg/kg b.w p.o. Eighteen male albino Sprague Dawley rats (180-200mg) were divided in to three groups. Group I- served as a vehicle treated control, group II- received 200mg/kg,b.w, p.o HENhance poly herbal formulation and group III- received standard drug sildenafil citrate 5mg/kg,b.w, p.o. for a period of 21 days. The female rats involved in mating were made receptive by hormonal treatment. The male treated groups of rats were observed for mounting behavior, mating behavior, orientation activity and for libido. At the end of experimental period the serum testosterone levels were observed. **Results:** The effective dose of HENhance was found to be 200mg/kg b.w from the acute toxicity studies. Oral administration of HENhance at a dose of 200mg/kg b.w significantly increased the serum testosterone, mounting behavior, orientation towards female rats, genital grooming, mounting frequency, intromission frequency and ejaculation latency along with decrease in mounting latency and intromission latency. **Conclusion:** This study suggests that HENhance was effective for sexual performance and safe alternative remedy for sexual disorders.

**KEYWORDS:** Aphrodisiac, Testosterone, Libido.

**INTRODUCTION**

Aphrodisiacs are the substances which are used to increase sexual activity and help in fertility. Sexual feelings are an inevitable part of life. The basic and fundamental purpose of sex and sexuality is the “continuation of progeny” and the survival of human race.<sup>[1]</sup> Sexual ignorance is a social disease and can only be resolved through comprehensive sex education, which can increase awareness and improve the environment. In fact, it is possible that with proper sex education, the number of unwanted pregnancies and sexually transmitted diseases would be reduced considerably.<sup>[2]</sup> Male sexual dysfunction (MSD) resulting in unsuccessful intercourse may adversely affect the personal and social life of the suffer couples and also contributes to infertility. A number of factors including psychological disturbances (performance anxiety, strained relationship, depression, stress, guilt and fear of sexual failure), deficiencies in sex hormones (testosterone deficiency), chronic diseases (diabetes, hypertension, atherosclerosis, venous leakage), neurological disorders (Parkinson’s disease, Alzhemier’s disease, spinal cord or nerve injury), side effects

associated with chronic use of drugs (anti-hypertensives, central agents, psychiatric medications, antiulcer, antidepressants, anti-androgens), life style related complications (chronic alcohol abuse, cigarette smoking) and aging are known to contribute to MSD.<sup>[3,4]</sup>

A human male may suffer from MSD at any stage of life but its risk increases with age. A population based study in US revealed that prevalence of MSD was 12 percent in those younger than 59 years, 22 percent in those 60 to 69 years of age, and 30 percent in those older than 69 years.<sup>[5]</sup> As per an estimate over 320 million people in the Westernized nations will be develop MSD by 2025.<sup>[6]</sup> The current epidemiological data suggests that MSD needs immediate medical intervention and newer therapeutic strategies are required for its management.

A number of treatment options are available for management of MSD. This option includes psychological and behavioural therapy, non-surgical treatments using constructive rings and vacuum pumps, surgical treatment such as penile prosthesis, penile implants and venous ligation, hormone replacement

therapy and intervention of chemotherapeutic agents.<sup>[7]</sup> The chemotherapeutic agents used for treatment of MSD are known as 'aphrodisiac'.

Research during the past two decades has an unfolded focus on impotence (erectile failure), premature ejaculation and male infertility. There are a number of prescription drugs which may act as sex stimulant and enhancing the sexual desire and activity in both men and women. Discovery of oral phosphodiesterase type 5 (PDE5) inhibitors particularly sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra) has revolutionized treatment of MSD.<sup>[8]</sup> Sildenafil citrate is the most prescribed PDE5, recommend in almost more than 70% of patients suffering from MSD. Mild to moderate headache, facial flushing, nasal congestion and dyspepsia are the most common adverse effects of PDE5 treatment.<sup>[9]</sup> Severe effects on PDE5 treatment have been reported in patients suffering from hypertension; hence careful clinical examination is a must before prescribing PDE5.

Although the use of allopathic medicines have shown significant improvement in treating sexual disorders, but the management therapies are too costly and not easily affordable at the same time there are large number of side effects. These include irregularities of the rhythm of the heart, suicidal tendencies, mental disorders and tremors. The use of synthetic aphrodisiacs results in the dilation of blood vessels in other parts of the body causing headache and fainting. Other side effects include facial flushing, stomach upset, blurred vision and sensitivity to light which usually occur at higher doses.<sup>[10]</sup> The side effects associated with these synthetic drugs necessitated search for safer and effective aphrodisiac agents especially of herbal origin. Medicinal plants represent an extraordinary reservoir of active ingredients.<sup>[11]</sup> Plant-derived chemicals are used to relieve sexual dysfunction and they have sex enhancing potentials. These phytochemicals increase libido, sexual potency and sexual pleasure.<sup>[12,13]</sup> The use of herbal medicine has become increasingly popular worldwide especially in the Asian and African countries.<sup>[14]</sup> Therefore in the present study we have evaluated the aphrodisiac activity of HENhance- a poly herbal formulation on male albino Sprague Dawley rats.

## MATERIALS AND METHODS

**Plant Material:** HENhance-polyherbal formulation developed by Suguna Food Pvt Ltd, Suguna Lifeherbs, Herbal Division, Coimbatore, Tamil Nadu, India.

## RESULT OF ACUTE TOXICITY STUDY

**Table. 1: Observation at 2000mg/kg dose of HENhance (single dose).**

Observation	At 30 min	4 h	24 h	48 h
Skin & fur	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal
Convulsion	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil

**Experimental Animals:** Healthy male albino rats (Sprague Dawley) weighing 180 to 200g and 6 female SD rats weighing 180-200g (for mating purpose). Animals and feed were procured from Adita Biosys Private Limited, Plot No- SPL-26, 2<sup>nd</sup> Stage, KSSIDC Industrial Area, Madhugiri Road, Antharasanahalli, Tumakur- 572106 CPCSEA Registration No: 1868/PO/Bt/S/16/CPCSEA. They were allowed free access to food pellets and water ad libitum. They were housed in well-ventilated polypropylene cages kept on the stainless steel racks maintained at relative humidity of 45-55%, 12h dark/light cycle under temperature 25°C ± 2°C, and were allowed for two weeks for acclimatization. The experimental protocols were approved by the Institutional Animal Ethics Committee (Ref No: PESCP/IAEC/28/2015.Date:29-12-2015) and conducted according to CPCSEA guidelines (CPCSEA Reg. No: 600/PO/Ere/S/02/CPCSEA), Govt. of India.

## Grouping of Animals for Acute Toxicity Studies

Eighteen animals were randomly divided into three groups as Group I, II, III containing six animals in each. The animals were selected in such a way that they were free from illness, injury, disease and kept in their cages for at least 10 days prior to dosing to allow for acclimatization to the laboratory conditions. Only those animals which are healthy and having weights 180-200gm were selected and maintained at standard laboratory conditions.

## Acute toxicity study

Prior to dosing, animals were fasted overnight before being weighed. Following the period of fasting, the fasted body weight of each animal was determined and the dose was calculated according to the body weight. Single animals were dosed in sequence usually at 48 h intervals. Using the default progression factor, doses will be selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550 and 2000mg (or 1.75, 5.5, 17.5, 55, 175,550, 1750, 5000mg for specific regulatory needs). Because no estimate of the substance's lethality is available, dosing was initiated at 175 mg/kg till 2000 mg/kg as recommended in OECD Guidelines 425.<sup>[15]</sup> And the LD50 was calculated by the changes in the observations for the main test at 2000 mg/kg body wt.

At the dose of 2000mg/kg no mortality was found (this observation was extended for further 14 days) from the above toxicity studies the effective dose (1/10<sup>th</sup> of 2000mg/kg) i.e...200 mg/kg was selected to evaluate the aphrodisiac activity of HENhance.

#### Grouping of Animals for aphrodisiac activity

Eighteen healthy male albino Sprague Dawley rats (180-200 g) were selected and grouped in to three each containing 6 animals.

**Group I-** served as a vehicle treated control.

**Group II-** received 200mg/kg.b.w, p.o HENhance poly herbal formulation for 21days.

**Group III-** received standard drug sildenafil citrate 5mg/kg.b.w, p.o for just one hour before of the commencement of the estimation. The experimental animals were maintained on regular rat food and drinking water *ad libitum*. The duration of the study was 21 days.

At the 20<sup>th</sup> day of the dosing period the female animals were artificially brought into oestrus (heat) (as the female rats allow mating only during the estrus phase) They were administered suspension of ethinyl oestradiol orally at the dose of 100 µg/animal, 48 hour prior to the pairing plus progesterone injected subcutaneously, at the dose of 1 mg/animal, 6 hour before the commencement of the experiment.<sup>[16]</sup>

The animals were observed for mounting behavior<sup>[17]</sup> regularly for 3h after a single dose of administration of HENhance.

At the end of the experimental period the animals were observed for mating behavior, orientation activity by the method of Sharma *et al*<sup>[18]</sup>, modified by Islam *et al*<sup>[19]</sup> and libido test by the method of Davidson<sup>[20]</sup> modified by Amin *et al*<sup>[21]</sup> followed by serum analysis, animals were anesthetized using light ketamine anaesthesia at a dose of

(40mg/kg b.w, i.p). The blood was collected by retro orbital puncture from all the animals and processed to obtain serum for serum testosterone analysis.<sup>[22]</sup>

#### Mounting behavior

To quantify mounting behavior, female rats were paired with males treated with a single dose of HENhance (200 mg/kg, p.o.) as well as with male rats in the control group and male rats treated with Sildenafil citrate (5mg/kg, p.o.). Animals were observed for 3 h and their behaviors were scored.<sup>[17]</sup>

#### Methods of scoring

Males were placed individually in a glass cage. After 15 min of acclimatization, a non-estrous female was introduced into the arena. The numbers of mounts were recorded during a 15-min observation period at the start of the 1<sup>st</sup> h. Then the female was separated for 105 min. Again the female was introduced and the numbers of mounts were observed for 15 min at the 3<sup>rd</sup> h. All the experiments were performed between 09:00 to 12:00 h during day time at room temperature, 26-27 °C.<sup>[23]</sup>

#### Procedure of scoring of mating behaviour

The animals were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment. The female animals were artificially brought into oestrus (heat)<sup>[24]</sup> by the Szechtman *et al*<sup>[25]</sup> method (as the female rats allow mating only during the oestrus phase) by administration of suspension of ethinyl estradiol orally at the dose of 100µg/animal, 48 h prior to the pairing. The most receptive females were selected for the study. The receptive female animals were introduced into the cages of male animals with 1 female to 1 male ratio and the occurrence of events and phases of mating was recorded. Their disappearance was also recorded. Later, the frequencies and sexual.



Fig. No. 01: Mounting behavior of rats.



Fig. No. 02: Mating behaviour of rats.

behavior phases were determined from cassette transcriptions: number of mounts before ejaculation or Mounting Frequency (MF), number of intromission before ejaculation or Intromission Frequency (IF), time

from the introduction of female into the cage of the male up to the first mount or Mounting Latency (ML), time from the introduction of the female up to the first intromission by the male or Intromission Latency (IL)<sup>[26]</sup>

**Orientation activity**-The test was carried out by the method of Sharma<sup>[18]</sup> et al, modified by Islam et al.<sup>[19]</sup> The orientation activity was carried out on the 21st day of treatment and was analyzed in three segments.

Orientation behavior of male rats was determined using following method of scoring

Orientation towards female – (1 for every sniffing and 2 for every licking)

Orientation towards self – (1 for every non-genital grooming and 2 for every genital grooming)

Orientation towards environment – (1 for every exploration, 2 for every rearing and 3 for every climbing).

**Libido test**- The test was carried out by the method of Davidson<sup>[20]</sup> modified by Amin *et al*<sup>[21]</sup> Each male rat was placed individually in a cage and the receptive female rats were introduced in the same cage. The animals were observed for Mounting Frequency (MF) on the evening of 21st day at 20:00 h. The penis was

exposed by retracting the sheath and 2% xylocaine ointment (procured from medical store) was applied 30, 15 and 5 min before starting the observations. The number of mountings, intromission and ejaculation were noted.

#### STATISTICAL METHODS

All data are expressed as the standard error of the mean. Comparisons among the control and treatment groups were made using analysis of variance (ANOVA) followed by a Bonferroni method of Statistics using the Graph pad prism statistical program. With all analyses, an associated probability (p value) of less than 5 % (P<0.05) was considered significant.

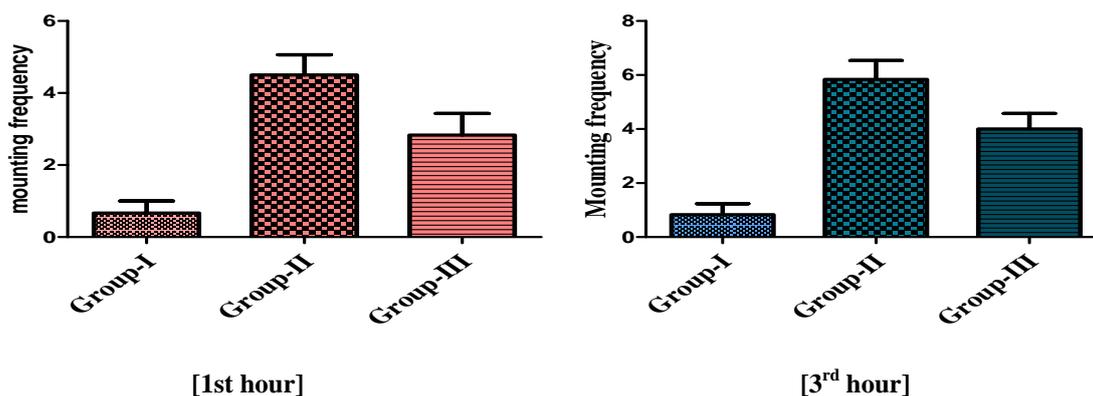
#### RESULTS

At the dose of 2000mg/kg no mortality was found (this observation was extended for further 14 days) from the above toxicity studies the effective dose (1/10<sup>th</sup> of 2000mg/kg) i.e...200 mg/kg was selected to evaluate the aphrodisiac activity of HENhance.

**Table. No. 2: Total serum testosterone estimation.**

Groups (n=6)	Treatment	Testosterone level (ng/dl)
I	Vehicle control	1.025± 0.4257
II	HENhance- poly herbal formulation	2.723±0.5665***
III	Standard drug (Sildenafil citrate)	2.118±0.6245*

Group-II rats were treated with HENhance-polyherbal formulation shows significant increase in (2.723±0.5665\*\*\*) testosterone content when compared with the Group-I normal control rat (1.025± 0.4257). Group-III rats were treated with standard aphrodisiac drug (Sildenafil citrate) also shows significant increase in (2.118±0.6245\*) testosterone level compared to the Group-I (Normal control group).



**Fig. 3: Effect of HENhance on Mounting behavior.**

Male rats (Group-II) treated with HENhance (200mg/kg) showed significant increase in the mounting behavior (4.500± 1.378\*\*\*) during 1<sup>st</sup> h after treatment as well as 3 h after treatment (5.833±1.722\*\*\*) when compared to control groups (Group-I) after 1<sup>st</sup>h (0.6667±0.8165) as well as after 3<sup>rd</sup> h (0.8333±0.9832) of the treatment. Male rats treated with standard drug (Sildenafil citrate) also shows significant result after 1<sup>st</sup> h (2.833±1.472\*) and after 3<sup>rd</sup> h (4.000±1.414\*\*) of the treatment as compared to Group-I.

The Group-II animals treated with HENhance at the dose level of 200mg/kg body weight markedly influenced the

orientation behavior, which showed more attraction towards female rats. The studies revealed significant increase in number of licking (6.667±3.502\*) and in the anogenital smelling (5.833± 1.941\*\*\*) of treated male rats towards receptive female comparable to the standard drug treated group of animals. The behavioral assessment of rats towards environment (exploration, rearing and climbing) was significantly decreased (0.8333±0.7528\*\*\*, 1.333±1.033\*\*\*, 0.5000±1.225\*\*\* respectively) in experimental animals and moderately decreased in standard group.

The studies on the genital grooming of male rats revealed that there was significant increase in genital grooming ( $9.333\pm 1.633^{***}$ ) in treated groups, while moderate decrease ( $2.000\pm 0.6325^{***}$ ) in non-genital grooming

was observed as compared with the control group. The standard drug also shows significant increase in genital grooming and decrease in non-genital grooming of male rats as compared to control group.

**Table. No. 3: Effect of HENhance on Orientation activity test.**

Gp n=6	Treatment	Orientation towards Female		Orientation towards self		Orientation towards environment		
		Sniffing	Licking	Non-genital grooming	Genital grooming	Exploration	Rearing	Climbing
I	Vehicle control	1.167±1.16 7	2.333± 1.506	7.833± 1.329	3.667± 1.506	7.000± 2.828	10.00± 2.191	5.500± 1.225
II	HENhance	5.833± 1.941***	6.667± 3.502*	2.000± 0.6325***	9.333± 1.633***	0.8333± 0.7528***	1.333± 1.033***	0.5000± 1.225***
III	Sildenafil citrate	3.833±1.72 2*	6.667± 2.066*	2.833± 1.169***	6.667± 2.066*	1.833± 2.137**	2.667± 1.033***	1.000± 1.549***

#### Effect of HENhance on libido

The result obtained in the test for libido shows that the Group-II (HENhance treated) showed significantly increased the Mounting Frequency (MF) ( $4.333\pm 1.633^{**}$ ) as compared to control group. The standard drug also significantly increased the MF

( $3.667\pm 1.211^*$ ) as compared to control animals. However the ejaculation was found absent in control group but present in both Group-II (HENhance treated) and group-III (standard drug). But the above result indicates that the increased libido activity was observed in Group-II.

**Table. No. 4: Effect of HENhance on libido.**

Groups (n=6)	Treatment	Mounting frequency (MF)	Intromission frequency (IF)	Ejaculation
I	Vehicle control	1.333±1.211	0.3333±0.5164	Absent
II	HENhance-	4.333±1.633**	2.500±1.378**	Present
III	Sildenafil citrate)	3.667±1.211*	1.167±0.7528	Present

**Table. No. 5: Effect of HENhance on Mating behavior.**

Groups (n=6)	Treatment	Mount latency (time in sec)	Mount frequency (No)	Intromission Latency (time in sec)	Intromission frequency (No)	Ejaculation latency (time in sec)
I	Vehicle control	133.3±47.19	2.333±2.066	82.50± 33.13	1.167±1.169	6.667±8.165
II	HENhance	50.00±27.57**	8.000±1.549***	20.83± 22.45**	4.333±1.633**	51.67±21.37**
III	Sildenafil citrate	63.33±22.51**	7.500±2.345***	31.67± 18.35**	4.500±1.517**	40.00±28.28*

The administration of HENhance-poly herbal formulation for 21 days to male rats resulted in remarkable increased in the sexual vigor of the male rats, as evidenced by the different sexual behavior parameters studied. The results of mating behavior test show that the Group-II (HENhance treated) significantly increased the Mounting frequency (MF) ( $8.000\pm 1.549^{***}$ ), Intromission frequency (IF) ( $4.333\pm 1.633^{**}$ ) and Ejaculation latency (EL) ( $51.67\pm 21.37^{**}$ ). Similarly it also causes significant reduction in the Mounting latency (ML) ( $50.00\pm 27.57^{**}$ ) and Intromission latency (IL) ( $20.83\pm 22.45^{**}$ ) in experimental animals as compared to the control group. Similarly, the standard drug also increased the MF ( $7.500\pm 2.345^{***}$ ), IF ( $4.500\pm 1.517^{**}$ ) and EL ( $40.00\pm 28.28^*$ ) as well as decreased the ML ( $63.33\pm 22.51^{**}$ ) and IL ( $31.67\pm 18.35^{**}$ ) in a highly significant manner as compared to control animals. The alteration in these parameters was statistically significant.

#### DISCUSSION

For the acute toxicity study of HENhance- poly herbal formulation the dosing was initiated at 175 mg/kg till 2000 mg/kg as recommended in OECD Guidelines 425. The LD50 was calculated by the changes in the observations for the main test at 2000 mg/kg body wt. At the dose 2000mg/kg no mortality was found (this observation was extended for further 14 days) so, 1/10 of this 200 mg/kg was selected as the median dose for this study.

The level of serum testosterone in HENhance-administered rats have shown significant increased level when compared to normal control rats. The standard drug Sildenafil citrate administered rats (group III) also shown significant increased level of testosterone. It is already reported that acute sildenafil treatment increases testicular interstitial fluid volume in the testes and also stimulates testosterone production from the leydig cells. Low testosterone affects male fertility by lowering sperm

count. The HENhance may increase the level of serum testosterone levels either by increasing the testicular interstitial fluid volume or by stimulating the leydig cells for the secretion of testosterone. The results suggest the fertility effect of HENhance.

Mammals exhibit sexually dimorphic behaviors that can be attributed to difference in brain structure and circulating hormone levels. Female rats displaying mounting behavior (mount with pelvic thrusts). Several reports suggested that, when a female mounts a male, the behavior may serve reproductive functions. The rats treated with HENhance have shown significant results when compared to normal rats suggesting its role in the reproductive function.

In male rats, latency for mount and intromission are considered as indicators of the sexual motivation, whereas intromission and ejaculation frequencies are considered as behavioral indication of sexual performance and facilitation. After treatment with the HENhance-polyherbal formulation (200mg/kg,b.w) there was a significant decrease in the latency for mount and intromission latencies indicating enhancing of sexual motivation, which was predominant at 21st day of observation. Similarly an increase in the number of ejaculations with an increase in the ejaculation latency indicated an increase in the sexual performance. Also it has a pronounced effect on sexual behavior shown by significant increase in Mounting frequency (MF) and Intromission frequency (IF) as compared to control. The MF and IF are considered the indices of both libido and potency. The significant increase in Ejaculation latency (EL) suggests that the experimental drug and standard drug prolonged the duration of coitus, which is an indicator of increase in sexual motivation.

Mounting frequency after penile anesthetization of rats is a reliable index of 'pure' libido and the penile reflexes of the rats are a good model of pure potency. The effect of the HENhance200mg/kg on libido was studied by assessing the MF after genital anaesthetization which does away with the reinforcing effect of genital sensation thus affording the study of pure libido or intrinsic sexual desire. During the experiment the test drug (HENhance) produced a significant increase in the MF of sexually normal male rats and in standard drug treated rats. Whereas, the MF was much reduced in control animal in which the penis had not been anaesthetized. However, the test for libido revealed that Intromission and Ejaculation were present in both standard and experimental groups of animals.

Administration of the HENhance at the dose of 200mg/kg body weight modified the rat orientation activities, which acts as a main determinant for measuring male sexual behavior. The effect of HENhance in the orientation activity study showed significantly more frequent and vigorous licking and anogenital sniffing of the receptive females sexually

experience treated male rats and their increased genital grooming as compared to control animals. All these indices indicate into significant increase in sexual motivation and vigor.

## CONCLUSION

Our findings suggest that HENhance- a polyherbal formulation developed by Suguna Food Pvt Ltd, Coimbatore, possesses the ability to increase aphrodisiac activity in male Sprague Dawley rats at the dose of 200mg/kg body weight without any noticeable adverse effect. In the present study it was observed that the total sexual behavior and the elevated testosterone level is more in the treated (HENhance) group when compared with that of standard group. Thus, this study suggests that HENhance was effective for sexual performance and safe alternative remedy for sexual disorders. The data obtained revealed that the action of the test drug was may be due to the influence on both sexual arousal and performance.

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