

**Butea Superba Rosb Extract Pharmacological Assessment for Aphrodisiac Effects**

Kushwaha A.K.,* Babar V.B.

School of pharmacy, SunRise University, Alwar, Rajasthan 301028, India

Email: ashok.cghs@gmail.com

Received 01 Oct 2023,

Accepted for publication 12 Dec 2023,

Published 30 Dec 2023

ABSTRACT

This study conducted a thorough assessment of the aphrodisiac effects of *Butea superba* Roxb extract, using multiple parameters to evaluate its influence on male reproductive functions. At first, the researchers compared the androgenic activity to testosterone. They found that the ethanolic extracts group showed a significant increase in body weight, testes, epididymis, seminal vesicles weight, and testosterone levels. These findings indicate notable androgenic effects of the extracts, which may be linked to the plant's androgenic properties. An analysis of sexual performance showed a decrease in the time it took for mounting and initiating sexual activity, along with an increase in the frequency of mounting and sexual activity in the group that received ethanolic extracts. The results of the study suggest an improvement in sexual activity among male rats, as evidenced by an increase in the penile erection index. Through behavioral analysis, it was observed that the group treated with the extract displayed increased exploratory behavior and mounting scores. This indicates an overall improvement in response to sexual stimulus. The testis sections were examined histologically and showed a significant improvement in spermatogenesis, similar to the effects of testosterone. The extracts showed an improvement in spermatogenic activity that varied based on the dosage. In addition, the study revealed that the group treated with ethanolic extracts had higher testosterone levels compared to the control and testosterone groups. This suggests a possible boost in testosterone production.

Keywords: Aphrodisiac Activity, Testosterone, Spermatogenesis, *Butea Superba*, Ethanolic Extract.**INTRODUCTION**

Sexual dysfunction is classified according to the various stages of sexual response, which include desire, arousal, ejaculation/orgasm, and resolution. Ancient Ayurveda recognized male sexual dysfunction long ago and developed a specialized field of medicine known as 'Vajikaran', dedicated to improving sexual performance. In the Ayurvedic system of medicine, Vajikaran rasayana is a term used to describe herbs or herbo-mineral preparations that enhance the qualities of rasa and increase its nutritional value. The advanced nature of this product helps to improve longevity, cognitive function, intelligence, sexual health, and youthful appearance. Vrishya rasayana is a

specialized type of rasayana that has a notable influence on the reproductive organs. It is advised for the purpose of maintaining fertility and improving sexual performance. It boosts sexual desire, enhances the quality of sperm, and promotes overall physical and psychological well-being. It is recommended to maintain youthful energy as one grows older.^[1]

Agents that elicit or enhance sexual desire and sexual performance are known as aphrodisiacs. The central nervous system regulates and controls sexual desire by processing sensory inputs from touch, smell, sound, and cognition. Sexual performance, in addition to sexual desire, is commonly known as performance or capability. Nonetheless, sexual dysfunction can occur even

*** Corresponding author:** Ashok Kumar Kushwaha

E-mail address: ashok.cghs@gmail.com

when there is a strong sexual desire. In such cases, the ability to engage in sexual activity depends on a neurovascular event that involves the hemodynamic processes responsible for penile erection [2].

Around 13–18% of couples face infertility, and there is growing evidence from clinical and epidemiological research suggesting a rising prevalence of male reproductive problems. A significant portion, specifically 51.2%, of instances where couples face infertility issues within their marriage can be traced back to male factors. Interestingly, in 39% of these cases, the males exhibited abnormal semen test results without any clear explanation. There are various genetic abnormalities that can contribute to male infertility, such as chromosomal aneuploidies, rearrangements, microdeletions, and single-gene issues. It affects not only the genes that control the creation of male reproductive cells, but also the complex system that oversees the development of male reproductive organs and body tissues. Various factors and hormones play a role in regulating the sexual behavior and activity of the male body. Testosterone, a vital hormone in male biology, plays a critical role in the growth and maturation of sperm [3-4].

Herbal medicine is based on the idea that plants contain natural compounds that can improve health and alleviate illnesses. There are a number of plants that have been discovered to have potential effects on sexual functions. These include various botanicals such as *Asparagus racemosus*, *Dactylorhiza hatagirea*, *Chlorophytum borivilianum*, *Curculigo orchioides*, *Tribulus terrestris*, *Aframomum melegueta*, *Eurycoma longifolia*, *Cnidium monnieri*, *Ferula harmonis*, *Mucuna pruriens*, *Lepidium meyenii*, and *Pausinystalia johimbe*, as well as *Passiflora incarnata*. These findings provide further evidence and present fresh opportunities. *Anacyclus pyrethrum DC*, commonly referred to as 'Akarkara' is highly regarded in the Ayurvedic tradition of Indian medicine for its tonic and rejuvenating

properties. The roots are also known for their potential effects on enhancing sexual desire and arousal [5].

Certain medicinal herbs, such as *Turnera diffusa* Wild (*Turnera diffusa*, *Turneraceae*), have been recognized for their potential to enhance sexual performance in individuals experiencing sexual dysfunction. These herbs are considered as a possible alternative therapy in this regard. *Tribulus terrestris* is a botanical species that has a long history of traditional use for various purposes, including addressing sexual impotence, edema, abdominal distention, cardiovascular ailments, renal disorders, and as a medicine for coughs. According to research, *Paederia foetida* has been found to have potential benefits such as improving sexual potency, increasing semen volume, enhancing physical strength, and promoting a youthful appearance [6]. The aim of this study was to examine the potential aphrodisiac effects of the extract derived from *Butea superba Roxb.*

MATERIALS AND METHODS

Animals

Albino rats (Wistar strain), regardless of gender, with a weight range of 100–120 g, were accommodated in a standardized environmental setting. They were provided with a standard diet and had unrestricted access to water at a controlled temperature of $24\pm2^{\circ}\text{C}$, following a day–night cycle from 06:00 h to 18:00 h. All animal experiments were conducted with prior approval from the institutional ethical committee of Dr. H.S. Gour University in Sagar, Madhya Pradesh, India.

Grouping of Animals

The animals were distributed into five groups, each comprising six rats. Group 1, designated as the control, received only the vehicle. Groups 2, 3, and 4 were orally administered with 50, 100, and 200 mg/kg/day of the ethanolic extract, respectively. Group 5, serving as the positive control for anabolic studies, received intramuscular injections of 0.5 mg/kg of testosterone suspended in arachis

oil twice weekly. Two groups of female rats were solely maintained on a standard diet. Prior to experimentation, all animals underwent a seven-day acclimatization period. Rats designated for physical behavior analysis were evaluated for their behavior and housed under observation in a glass cage measuring 60×50×40 cm. To distinguish between groups, the animals were marked using picric acid with distinct markings assigned to each group [7-8].

Preparation of Test and Standard Drug Suspension

Suspension of drug was prepared by suspending the ethanolic extract of *Butea superba* Roxb in 2% tween 80 suspension solution. Testosterone suspended in arachis oil was prepared giving a suspension of 0.5 mg/kg.

Dose and Route of Administration

Ethanolic extract of *Butea superba* Roxb was given in a suspended form containing 50,100 and 200 mg/kg body weight. The suspended extracts were given orally by metal canula [9]. Testosterone dose was given as 0.5 mg/kg body weight twice weekly according to IP,1996.

Statistical Analysis

Data were expressed as mean± standard error of mean and statistical analysis was carried out using Dunnett test. The values were calculated and analyzed using Graphpad Instat 3.06 software run on Windows XP (Microsoft Corp.).

Parameters for Evaluation of Aphrodisiac Activity

Sexual Behavior Analysis

The assessment of sexual behavior is crucial for determining the sexual potency of herbal drugs known for their proven sexual vigor and aphrodisiac properties. The impact on the sexual behavior of male rats was evaluated at 0, 15, and 28 days of treatment. In brief, a male rat was introduced into observation glass chambers for a 5-minute acclimatization period to the cage

environment. A sexually receptive female rat was then gently introduced from one side of the chamber as a stimulus. The observations included recording sexual behavior parameters such as mount latency (ML) and mount frequency (MF). ML was calculated as the time from the introduction of the female to the occurrence of the first mount, while MF was the total number of mounts observed within a 30-minute period [10-11].

Intromission latency (IL) was determined as the time taken for the first intromission (insertion of the penis into the female rat's vagina) after introducing the female into the cage. Intromission frequency was recorded as the total number of intromissions within 30 minutes. Penile erection, as per the method described by Bennassi-Benelli et al. and modified by Islam et al., was noted when copulatory movements were observed in males in the absence of a female rat, culminating in the male rat bending down to lick its fully erect penis, leading to ejaculation. The penile erection index was calculated by multiplying the percentage of rats exhibiting at least one episode of penile erection during a one-hour observation by the mean number of penile erections. These parameters collectively provided insights into the sexual behavior and potency of the herbal drug under investigation [12]. Penile erection index (PEI) was calculated as:

$$\text{PEI} = \% \text{ of rats exhibiting penile erection} \times \text{Mean number of erections}$$

Orientational Behavior Analysis

The impact of extracts on behavioral aspects was assessed by evaluating three distinct parameters: self-exploratory behavior, which encompassed rearing, self-licking, and anogenital sniffing; environmental exploration, which included activities such as exploration, roaming, and climbing; and non-self exploratory behavior, involving mounting over females, licking, and anogenital sniffing. Recordings of these behaviors were conducted on days 0, 15, and 28 following the initiation of treatment [13].

Anabolic Effect Determination

The animals in all groups received the designated dose for a duration of 28 days. The weights of the animals were measured and recorded on days 0, 15, and 28. After the 28-day treatment period, the body weights of the animals were recorded, following which three animals from each group were euthanized by decapitation. The testes, epididymis, seminal vesicles, and prostate glands were meticulously removed and weighed [14].

Histological Studies

Following 28 days of treatment for animals in all respective groups, the testes were dissected out, and approximately 5 μ m thick testicular sections were fixed in Bouin's fixative. Subsequently, the sections were dehydrated using varying percentages of ethanol and stained with hematoxylin and eosin. Microscopic evaluation of the thin sections was carried out, and variations in histoarchitecture were meticulously recorded [15].

Serum Hormone Level Detection

The serum concentration of total testosterone was determined using a double antibody ELISA kit from Eiagen Testosterone kit, Italy, following the standard protocol provided in the assay kit. This method is commonly employed to measure hormone levels, detecting testosterone, estrogen, cortisol, and thyroid levels. It is important to specify the gender when ordering a hormone test, as women's and men's tests will examine different levels of sex hormones. In another aspect of the study, 21-day-old rats underwent immunization seven times with FSH peptides linked with Keyhole Limpet Hemocyanin (KLH) in the experimental group, while the control group received KLH alone, with immunizations administered every 2 weeks. The levels of Luteinizing hormone (LH) and inhibin B in the immunized rat sera were measured using enzyme-linked immunosorbent assay (ELISA). The apoptosis of spermatogenic cells in the testis was detected using the in situ end labeling method

(TUNEL), and the mRNA expression of Bax, Bcl-2, and Caspase-3 in the testis was determined using fluorescent Quantitative PCR [16].

RESULTS AND DISCUSSION

Effect of Ethanolic extract of *Butea superba* on sexual behavioral

On the 15th day, mount latency significantly decreased in the ethanolic extract 200 mg group (161.1 ± 4.16) and the testosterone group (157.1 ± 3.06) compared to the control group. Subsequent observations on the 28th day of extract administration revealed a considerable decrease in mount latency in the ethanolic extract 200 mg group (139.6 ± 4.45) and the testosterone group (141.3 ± 4.80) compared to the control group (203.1 ± 3.97). These results indicate that the ethanolic extract at a dose of 200 mg exhibited the maximum reduction in mount latency.

Table 1: Mount Latency (time in seconds)

Group	Dose (mg/kg)	0 days	15 days of Treatment	28 days of Treatment
Control	-	201.3 ± 3.98	207.1 ± 4.35	203.1 ± 3.97
Ethanolic Extract	50	199.1 ± 4.62	$181.3 \pm 3.07^*$	$173.5 \pm 4.37^*$
Ethanolic Extract	100	202.5 ± 3.39	$170.6 \pm 4.45^*$	$141.6 \pm 3.93^*$
Ethanolic Extract	200	198.6 ± 4.96	$161.1 \pm 4.16^{**}$	$139.6 \pm 4.45^{**}$
Testosterone Group	0.5	201.0 ± 4.60	$157.1 \pm 3.06^{**}$	$141.3 \pm 4.80^{**}$

All values are expressed as mean \pm S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control

On the 15th day, mount frequency significantly increased in the ethanolic extract 200 mg group (10.16 ± 0.75) and the testosterone group (7.66 ± 0.51) compared to the control group. Subsequent observations on the 28th day of extract administration revealed a considerable increase in

mount frequency in the ethanolic extract 200 mg group (14.33 ± 0.81) and the testosterone group (11.83 ± 0.75) compared to the control group (4.33 ± 0.51). These results suggest that the ethanolic extract at a dose of 200 mg exhibited the maximum increase in mount frequency.

Table 2: Mount Frequency

Group	Dose (mg/kg)	0 days	15 days of Treatment	28 days of Treatment
Control	-	3.66 ± 0.51	4.0 ± 0.63	4.33 ± 0.51
Ethanolic Extract	50	4.83 ± 0.75	7.0 ± 0.63	$10.33 \pm 0.51^{**}$
Ethanolic Extract	100	4.66 ± 0.81	$10.33 \pm 0.81^{**}$	$12.83 \pm 0.75^{**}$
Ethanolic Extract	200	3.83 ± 0.75	$10.16 \pm 0.75^{**}$	$14.33 \pm 0.81^{**}$
Testosterone Group	0.5	3.83 ± 0.75	$7.66 \pm 0.51^*$	$11.83 \pm 0.75^{**}$

All values are expressed as mean \pm S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control.

On the 15th day, intromission latency significantly decreased in the ethanolic extract 200 mg group (276.3 ± 3.77) and the testosterone group (284.5 ± 5.75) compared to the control group. Subsequent observations on the 28th day of extract administration revealed a considerable decrease in intromission latency in the ethanolic extract 200 mg group (251.3 ± 4.41) and the testosterone group (260.8 ± 4.35) compared to the control group (317.5 ± 4.32). These results suggest that the ethanolic extract at a dose of 200 mg exhibited the maximum decrease in intromission latency.

Additionally, intromission frequency significantly increased on the 15th day in the ethanolic extract 200 mg group (3.16 ± 0.75) and the testosterone group (2.33 ± 0.81) compared to the control group. Subsequent observations on the 28th day of extract administration revealed a considerable increase in

intromission frequency in the ethanolic extract 200 mg group (4.83 ± 1.32) and the testosterone group (3.95 ± 0.98) compared to the control group (1.50 ± 0.54). These results suggest that the ethanolic extract at a dose of 200 mg exhibited the maximum increase in intromission frequency.

Table 3: Intromission Latency (time in Secs)

Group	Dose (mg/kg)	0 days	15 days of Treatment	28 days of Treatment
Control	-	320.6 ± 4.41	316.5 ± 4.50	317.5 ± 4.32
Ethanolic Extract	50	312.8 ± 5.63	304.5 ± 3.61	$272.8 \pm 6.55^*$
Ethanolic Extract	100	317.5 ± 5.89	$281.6 \pm 6.94^{**}$	$263.3 \pm 4.13^{**}$
Ethanolic Extract	200	323.1 ± 4.35	$276.3 \pm 3.77^{**}$	$251.3 \pm 4.41^{**}$
Testosterone Group	0.5	318.6 ± 4.41	$284.5 \pm 5.75^*$	$260.8 \pm 4.35^{**}$

All values are expressed as mean \pm S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control

Table 4: Intromission Frequency

Group	Dose (mg/kg)	0 days	15 days of Treatment	28 days of Treatment
Control	-	1.16 ± 0.40	1.33 ± 0.51	1.50 ± 0.54
Ethanolic Extract	50	1.83 ± 0.75	2.06 ± 0.81	$3.16 \pm 0.75^*$
Ethanolic Extract	100	2.0 ± 0.63	$2.83 \pm 0.98^*$	$4.16 \pm 0.98^{**}$
Ethanolic Extract	200	2.16 ± 0.75	$3.16 \pm 0.75^{**}$	$4.83 \pm 1.32^{**}$
Testosterone Group	0.5	1.16 ± 0.40	$2.33 \pm 0.81^*$	$3.95 \pm 0.98^{**}$

All values are expressed as mean \pm S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control

Effect of extracts of *Butea superba* on penile Erection Index in male rats

Penile Erection Index (PEI) was increased in the ethanolic extracts treated Groups on 28th days of treatment. In control Group the value for PEI was (28.64 ± 2.31), the value for PEI in the ethanolic extracts 200 mg (76.50 ± 3.38) and the value of PEI was increase in testosterone (55.00 ± 2.80) treated group. The maximum improvement in the ethanolic extracts 200 mg groups was on 28th days of observation as compared to control group.

Table 5: Determination of Penile Erection Index in rats

Group	Dose (mg/kg)	0 days	15 days of Treatment	28 days of Treatment
Control	-	17.65 ± 1.03	19.98 ± 2.44	19.64 ± 2.31
Ethanolic Extract	50	9.99 ± 1.63	25.66 ± 3.65	$50.00 \pm 2.63^{**}$
Ethanolic Extract	100	19.98 ± 1.63	$55.00 \pm 1.87^{**}$	$73.26 \pm 3.67^{**}$
Ethanolic Extract	200	9.99 ± 2.33	$54.37 \pm 2.42^{**}$	$76.50 \pm 3.38^{**}$
Testosterone Group	0.5	8.30 ± 1.16	$28.64 \pm 1.32^*$	$55.00 \pm 2.80^{**}$

All values are expressed as mean \pm S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control

Effect of extracts of *Butea superba* on Orientation Activities in male rats

Orientation activities studies showed that upon treatment with extract and testosterone there was a significant increase in attraction of male towards female indicated by enhanced licking and anogenital sniffing as compared to control. The attraction towards environment was evidently more in case of extract treated group when compared to control. There is also increase in attraction towards self and genital grooming of male rats treated that is comparable to standard.

Ethanol extract 200 mg show maximum orientation activities than compared to all groups. It terms of various observation taken at different day intervals it was observed that in case of 50, 100 and 200 mg of ethanolic extracts treated groups the increase in orientation activity was increased from the 15th day of observation this improvement continued till the 28th day of observation.

Table 6: Effect of ethanolic extracts of *Butea superba* on Orientation activity

Mean activity Score Towards Environment (Exploration, Raring and Climbing)

Group	Dose (mg/kg)	0 days	15 days of Treatment	28 days of Treatment
Control	-	11.16 ± 0.75	11.33 ± 1.21	11.5 ± 1.04
Ethanolic Extract	50	11.83 ± 0.98	$13.16 \pm 0.75^{**}$	$17.66 \pm 1.21^{**}$
Ethanolic Extract	100	12.33 ± 1.36	$14.66 \pm 1.50^{**}$	$21.83 \pm 0.98^{**}$
Ethanolic Extract	200	12.50 ± 1.22	$14.83 \pm 1.16^{**}$	$22.66 \pm 0.81^{**}$
Testosterone Group	0.5	11.33 ± 1.96	13.16 ± 1.47	$19.5 \pm 1.87^{**}$

All values are expressed as mean \pm S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control

Table 7: Mean activity Score Towards Self (Nongenital grooming and Genital grooming)

Group	Dose (mg/kg)	0 days	15 days of Treatment	28 days of Treatment
Control	-	7.83 ± 0.75	7.50 ± 1.37	7.66 ± 1.21
Ethanolic Extract	50	8.16 ± 0.98	11.83 ± 0.75	$13.66 \pm 1.50^*$
Ethanolic Extract	100	8.50 ± 0.83	$13.0 \pm 1.09^*$	$13.83 \pm 1.72^{**}$

Ethanol Extract	200	8.0 ± 0.89	14.16 ± 0.98**	15.66 ± 1.36**
Testosterone Group	0.5	7.83 ± 1.47	15.16 ± 0.75**	14.66 ± 1.21**

All values are expressed as mean ± S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control

Effect of extracts of *Butea superba* on body and Organ Weight

The effect of the ethanolic extracts of *Butea superba* on body and organ weight increases. Body weight and organ weight summarized in table 5.10 and 5.11. 50, 100, and 200 mg ethanolic extracts of *Butea superba* and testosterone treated group resulted in an increase the body weight (149.5 ± 3.45), (158.1 ± 4.79), (165.5 ± 2.16), and (161.3 ± 3.32) respectively with compare to control (121.1 ± 5.11) on 28th day of treatment.

The administration of ethanolic extract at a dose of 50, 100 and 200 mg respectively resulted in an increase the weight of testis (1017±22.3), (1023±22.6), and (1034±24.0mg), epididymis (798±16.5), (809±17.0), and (818±16.0mg) seminal vesicles (433±21.9), (444±22.3) and (452±22.3mg) and prostate glands (297±14.5), (304±15.5) and (307±15.7mg), whereas the administration of testosterone also significant increase in weight of testis to (1024±21.5mg) and appreciable increase in the weight of epididymis (808±14.0mg), seminal vesicle (443±21.3mg), prostate glands (313±16.1mg) and as compared to normal control group.

Table 8: Effect of ethanolic extracts of *Butea superba* on body weight of rats. Body weight (gm).

Group	Dose (mg/kg)	0 days	15 days of Treatment	28 days of Treatment
Control	-	109.0 ± 6.16	113.6 ± 5.95	121.1 ± 5.11
Ethanol	50	101.5 ±	121.5 ±	149.5 ±

Extract		2.42	3.20**	3.45**
Ethanol Extract	100	102.1 ± 2.56	137.6 ± 5.20**	158.1 ± 4.79
Ethanol Extract	200	102.33 ± 2.80	141.8 ± 2.22**	165.5 ± 2.16**
Testosterone Group	0.5	103.8 ± 4.26	138.5 ± 2.42**	161.3 ± 3.32**

All values are expressed as mean ± S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control

Table 9: Effect of ethanolic extracts of *Butea superba* on organ weight of rats. Organ Weight (mg/100g b.w).

Group	Weight of testes	Weight of Epididymides	Weight of seminal vesicles	Weight of prostate
	28 days	28 days	28 days	28 days
Control	976±18.0	748±15.7	415±19.3	284±14.5
Ethanol Extract 50	1017±22.3	798±16.5*	433±21.9	297±14.5
Ethanol Extract 100	1023±22.6*	809±17.0**	444±22.3*	304±15.5
Ethanol Extract 200	1034±24.0**	818±16.0**	452±22.3*	307±15.7
Testosterone Group 0.5	1024±21.5*	808±14.0**	443±21.3*	313±16.1

All values are expressed as mean ± S.E.M., n=6; P*<0.05 and P**<0.01 considered significant as compared to control

Effect of extracts of *Butea superba* on Testosterone level

There are dose dependent increases in serum testosterone concentration. Extract administration which produced significant increase in serum testosterone concentration.

Histological Examination

Animals in the control group exhibited a normal histological texture in the testis section. Within a certain spectrum, the diameter of seminiferous

tubules varied. The tubules with the greatest diameter were uncommon and well within the expected range. The dimensions and morphology of the cuboidal germinal epithelium were typically observed. Sertoli cells exhibited a profusion of cytoplasmic processes of typical dimensions. Serotoli cells encased spermatozoa, which exhibited typical cytoplasmic granulation. Leydig's cells possessed typical-sized nuclei. The luminal portion of the tubule contained bundles of spermatozoa in a normal number. Spermatozoa characterized by a long tail and a small, distinct head were more apparent.

Spermatogenesis increases substantially after 28 days of treatment with ethanolic extract at doses of 50, 100, and 200 mg, in comparison to the control group. The experimental group that received the extract exhibited significant improvements in testis weight and histological changes. The extract-treated groups exhibited greater weight and size of the testis, which resulted in nearly all seminiferous tubules demonstrating an increased diameter. It appeared that the cells of the germinal epithelium were hyperactive. A considerable variety of cells at various phases of spermatogenesis were readily observable. Each lumen of the seminiferous tubule contained an immense quantity of spermatozoa. Proliferating Sertoli cells were nutrient-dense and highly processed, as indicated by their densely granulated cytoplasm. Nearly every Ledig cell exhibited hypertrophy, characterized by an enlarged nucleus and darkly pigmented cytoplasm. The observed increase in cellular and nuclear volume was highly indicative of steroid synthesis occurring either directly or indirectly as a result of the drug. Sperm packages filled nearly every tubule to capacity. Spermatids were discovered strewn among spermatozoa in certain tubules. Mild dilation of the blood vessels in the testis was observed.

The histoarchitecture of the group treated with testosterone was comparable to that of the group treated with ethanolic 200. Compared to the control group, increased spermatogenesis was

demonstrated by an increase in the number of spermatozoa in seminiferous tubules and spermatogenic elements. The testosterone-treated group exhibited a notable increase in leydig cells and interstitial cells in comparison to the control group. Additionally, the dense clustering indicates that testosterone may play a role in promoting the vascularization of testicular tissues.

CONCLUSION

The study evaluated the aphrodisiac activity of *Butea superba* extracts in rats. The androgenic activity of the extracts was compared to testosterone, which is responsible for the androgenic effect. The ethanolic extracts group showed a significant increase in body weight, testes, epididymis, seminal vesicles weight, and testosterone levels compared to the control and testosterone groups. This suggests that the extracts enhance sexual differentiation male organs.

The administration of the extract also improved sexual performance evaluation. The mount latency and intromission latency time were reduced in the extract group, while the effects were also increased in the testosterone-administered group. The results indicate an improvement in sexual performance and activity time in male rats. The *Butea superba* extracts also increased the penile erection index in male rats. The key events in the erection process are relaxation of the penile arterial system, which enhances blood pressure in the corpora cavernosa, and relaxation of the trabecular smooth muscle, which allows lacunar spaces to expand and cavernosal venous outflow to be reduced by compression of the veinules against the tunica albuginea. The histology of the testis revealed a marked effect of extracts and testosterone on spermatogenesis. The extracts showed an increase in testosterone level in the testis in dose-dependent manner. In conclusion, *Butea superba* has a positive influence on overall sexual behavior, anabolic activity, sexual performance, vigor, body strength, and youthful glow. Further experiments will include fractionation of the extract,

identification of therapeutic bioactive compounds, and determination of its pharmacological action for aphrodisiac activity..

Conflict of Interest- No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

Acknowledgement: We would like thank to School of pharmacy, SunRISE University, Alwar for providing all facilities to conduct the research work.

Funding Resources: None

REFERENCES

1. Rullyansyah S, Muzakky F, Samlan K, Hanistya R, Maulidiyanti ETS. Phytochemical screening and evaluation of red ginger extracts on aphrodisiac activity. *Gac Med Caracas* [Internet]. 2022;130(Supl. 1). Available from: <http://dx.doi.org/10.47307/gmc.2022.130.s1.26>
2. Asfour HZ, Alhakamy NA, Fahmy UA, Ahmed OAA, Rizg WY, Felimban RI, et al. Zein-stabilized nanospheres as nanocarriers for boosting the aphrodisiac activity of icariin: Response surface optimization and in vivo assessment. *Pharmaceutics* [Internet]. 2022;14(6). Available from: <http://dx.doi.org/10.3390/pharmaceutics14061279>
3. Praptiwi, Wulansari D, Kamitakahara H, Takano T, Munawarah AU, Agusta A. Evaluation of aphrodisiac activity of yellow ginseng (*Rennelia elliptica* Korth.) root ethanol extract in male DDY mice (*Mus musculus*). In: AIP Conference Proceedings. AIP Publishing; 2022.
4. Kusumawati I, Mahatmaputra S, Hadi R, Rohmania R, Rullyansyah S, Yusuf H, et al. Aphrodisiac activity of ethanolic extracts from the fruits of three pepper plants from Piperaceae family. *J Farm Dan Ilmu Kefarmasian Indones* [Internet]. 2021;8(2):194. Available from: <http://dx.doi.org/10.20473/jfiki.v8i22021.194-199>
5. Gayathri A G, Mruthunjaya K. Aphrodisiac activity of nutraceuticals. *Int J Res Pharm Sci* [Internet]. 2020;11(3):3878–88. Available from: <http://dx.doi.org/10.26452/ijrps.v11i3.2572>
6. Singh SK, Jain AP, Tandon M. Investigations on *Citrullus colocynthis* seeds for aphrodisiac activity in male albino rats. *Adv Pharm J* [Internet]. 2020;5(3):119–22. Available from: <http://dx.doi.org/10.31024/apj.2020.5.3.6>
7. Aydogan F, Baykan S, Soliman GA, Yusufoglu H, Bedir E. Evaluation of the potential aphrodisiac activity of sesquiterpenoids from roots of *Ferula hubermannii* Peşmen in male rats. *J Ethnopharmacol* [Internet]. 2020;257(112868):112868. Available from: <http://dx.doi.org/10.1016/j.jep.2020.112868>
8. Choudhury MRA, Basak M. Ethno medicinal plants used for aphrodisiac activity in North-East, India. *J Pharm Res Int* [Internet]. 2021;314–22. Available from: <http://dx.doi.org/10.9734/jpri/2021/v33i38b32129>
9. Gupta M, Mondal AK. Clinical evaluation of aphrodisiac activity of a novel ayurvedic formulation for treatment of male sexual disorders. *Int J Res Med Sci* [Internet]. 2020;8(7):2515. Available from: <http://dx.doi.org/10.18203/2320-6012.ijrms20202888>
10. Vasantrao US, Biyani KR. Evaluation of Preclinical Aphrodisiac Activity of *Flueggea leucopyrus* Willd. Leaves. *Curr Bioact Compd* [Internet]. 2020;16(3):335–41. Available from: <http://dx.doi.org/10.2174/1573407214666181105115510>
11. Kadzo L. Aphrodisiac Activity of Pomegranate (*Punica granatum* L.) fruit

- extract on the sexual function in rats. *Discov Phytomedicine* [Internet]. 2020;7(2). Available from: <http://dx.doi.org/10.15562/phytomedicine.2020.121>
12. Du Q, Huang Y-H, Bajpai A, Frosig-Jorgensen M, Zhao G, Craik DJ. Evaluation of the in vivo aphrodisiac activity of a cyclotide extract from *Hybanthus enneaspermus*. *J Nat Prod* [Internet]. 2020;83(12):3736–43. Available from: <http://dx.doi.org/10.1021/acs.jnatprod.0c01045>
13. Aphrodisiac activity of vanari gutika formulated using milk obtained from jersey cow (*Bos taurus*), indigenous cow (*Bos indicus*) and buffalo (*Bubalus bubalis*). *Ind J Tradit Knowl* [Internet]. 2020;19(3). Available from: <http://dx.doi.org/10.56042/ijtk.v19i3.41480>
14. Oboh G, Adebayo AA, Ademosun AO, Abegunde OA. Aphrodisiac effect of *Hunteria umbellata* seed extract: Modulation of nitric oxide level and arginase activity in vivo. *Pathophysiology* [Internet]. 2019;26(1):39–47. Available from: <http://dx.doi.org/10.1016/j.pathophys.2018.11.003>
15. Borkar SS, Mahapatra DK, Usare S. Reviewing the aphrodisiac activity of *lepedium meyenii* plant in modern contexts. In: *Natural Products Pharmacology and Phytochemicals for Health Care*. Includes bibliographical references and index.: Apple Academic Press; 2020. p. 41–54.
16. Karunanithi M, David Raj C, Jegadeesan M, Kavimani S. Phytochemical analysis and comparative aphrodisiac activity of four species of *Mucuna*. In: *Phytopharmaceuticals and Drug Delivery Approaches*. Avid Science; 2019. p. 02–29.