



Pharmacological evaluation of aphrodisiac activity of *Butea superba* Roxb Extract

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ABSTRACT

This study undertook a comprehensive evaluation of the aphrodisiac activity of Butea superba Roxb extract, utilizing various parameters to assess its impact on male reproductive functions. Initially, androgenic activity was compared to testosterone, showcasing a notable increase in body weight, testes, epididymis, seminal vesicles weight, and testosterone levels in the ethanolic extracts group. This suggests significant androgenic effects of the extracts, potentially attributed to the plant's androgenic properties. Sexual performance evaluation demonstrated a reduction in mount latency and intromission latency times, coupled with an increase in mount and intromission frequencies in the ethanolic extracts group. These findings indicated an enhancement of sexual activity in male rats, further supported by an increased penile erection index. Behavioral analysis revealed heightened exploratory behavior and mounting scores in the extract-treated group, highlighting an overall improvement in sexual stimulus. Histological examination of testis sections demonstrated enhanced spermatogenesis, comparable to the effects of testosterone. The extracts exhibited a dose-dependent improvement in spermatogenic activity. Additionally, testosterone levels were found to be higher in the ethanolic extracts group compared to control and testosterone groups, indicating a potential increase in testosterone production.

Keywords: *Butea superba*, ethanolic extract, aphrodisiac activity, testosterone, Spermatogenesis.

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INTRODUCTION

Sexual dysfunction is categorized based on the many stages of sexual response, including desire (hypoactive sexual desire disorder/sexual aversion), arousal (male erectile disorder), ejaculation/orgasm (male orgasmic disorder, premature ejaculation), and resolution. Ayurveda identified male sexual dysfunction millennia ago and established a distinct branch of medicine called 'Vajikaran', which focuses on enhancing sexual performance. In the Ayurvedic system of medicine, Vajikaran rasayana refers to herbs or herbo-mineral preparations that improve the properties of rasa and boost its nutritional content. The enhanced essence contributes to achieving lifespan, cognitive abilities, intellect, absence of sexual diseases, and young appearance. Vrishya rasayana is a specific kind of rasayana that has a significant impact on the sexual organs. It is recommended for the purpose of preserving fertility and enhancing sexual performance. It enhances libido, improves sperm quality, and contributes to general physical and psychological well-being. It is advised for keeping young vitality as one ages [1].

Aphrodisiacs are agents that elicit or enhance sexual desire and sexual performance. The central nervous system governs and manages sexual desire by integrating sensory inputs from touch, smell, sound, and cognition. Sexual performance, apart from sexual desire, is often referred to as performance or capability. However, sexual dysfunction may manifest even when there is a strong sexual desire. In such cases, the ability to act sexually relies on a neurovascular event that involves the hemodynamic processes responsible for penile erection [2].

Approximately 13–18% of couples experience infertility, and mounting evidence from clinical and epidemiological research indicates an increasing prevalence of male reproductive issues. 51.2% of cases of marital infertility may be attributed to male causes, and in 39% of these cases, the males had abnormal semen tests for unknown reasons. Male infertility may result from many genetic abnormalities, including significant chromosomal aneuploidies, rearrangements, microdeletions, and single-gene problems. It impacts not just the genes that regulate the production of male reproductive cells, but also the intricate system responsible for male reproductive organ development and the development of male body tissues. The male body's sexual behavior and activity are regulated by several elements and hormones.

Testosterone, the primary male sex hormone, is crucial for the development and maturation of sperm [3-4].

Herbal medicine operates on the principle that plants include inherent compounds that may enhance well-being and ease ailments. Several botanicals, including *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 incarnate*, have been found to potentially affect sexual functions. These findings support previous claims and offer new possibilities. *Anacyclus pyrethrum* DC, generally known as 'Akarkara', is widely acknowledged in the Ayurvedic school of Indian medicine as a tonic and rejuvenator. The roots are also regarded as aphrodisiacs and sexual stimulants [5].

Medicinal herbs known for their aphrodisiac properties, including *Turnera diffusa* Wild (*Turnera diffusa*, *Turneraceae*), are seen as a potential alternative therapy for improving sexual performance in individuals with sexual dysfunction. *Tribulus terrestris* is a botanical species that has been traditionally used for many purposes, such as addressing sexual impotence, edema, abdominal distention, cardiovascular ailments, renal disorders, and as a medicine for coughs. *Paederia foetida* is said to enhance sexual potency, augment semen volume, boost physical strength, and impart a young radiance [6]. The objective of this research was to investigate the aphrodisiac activity of *Butea superba* Roxb Extract.

MATERIAL 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 $24\pm 2^{\circ}\text{C}$, 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 \pm 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: PEI = % of rats exhibiting penile erection × Mean number of erections

Oriental Behavioral 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 5µm 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 ± 3.07* | 173.5 ± 4.37* |
| Ethanolic Extract | 100 | 202.5 ± 3.39 | 170.6 ± 4.45* | 141.6 ± 3.93* |
| Ethanolic Extract | 200 | 198.6 ± 4.96 | 161.1 ± 4.16** | 139.6 ± 4.45** |
| Testosterone Group | 0.5 | 201.0 ± 4.60 | 157.1 ± 3.06** | 141.3 ± 4.80** |

All values are expressed as mean ± 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 Seconds)

| 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 |
| Ethanollic Extract | 50 | 9.99 ± 1.63 | 25.66 ± 3.65 | 50.00 ± 2.63** |
| Ethanollic Extract | 100 | 19.98 ± 1.63 | 55.00 ± 1.87** | 73.26 ± 3.67** |
| Ethanollic Extract | 200 | 9.99 ± 2.33 | 54.37 ± 2.42** | 76.50 ± 3.38** |
| Testosterone Group | 0.5 | 8.30 ± 1.16 | 28.64 ± 1.32* | 55.00 ± 2.80** |

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 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. Ethanolic 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 |
| Ethanollic Extract | 50 | 11.83 ± 0.98 | 13.16 ± 0.75** | 17.66 ± 1.21** |
| Ethanollic Extract | 100 | 12.33 ± 1.36 | 14.66 ± 1.50** | 21.83 ± 0.98** |
| Ethanollic Extract | 200 | 12.50 ± 1.22 | 14.83 ± 1.16** | 22.66 ± 0.81** |
| Testosterone Group | 0.5 | 11.33 ± 1.96 | 13.16 ± 1.47 | 19.5 ± 1.87** |

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 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 |
| Ethanollic Extract | 50 | 8.16 ± 0.98 | 11.83 ± 0.75 | 13.66 ± 1.50* |
| Ethanollic Extract | 100 | 8.50 ± 0.83 | 13.0 ± 1.09* | 13.83 ± 1.72** |
| Ethanollic 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 50,100and 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 |
| Ethanolic Extract | 50 | 101.5 ± 2.42 | 121.5 ± 3.20** | 149.5 ± 3.45** |
| Ethanolic Extract | 100 | 102.1 ± 2.56 | 137.6 ± 5.20** | 158.1 ± 4.79 |
| Ethanolic 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 |
| Ethanolic Extract 50 | 1017±22.3 | 798±16.5* | 433±21.9 | 297±14.5 |
| Ethanolic Extract 100 | 1023±22.6* | 809±17.0** | 444±22.3** | 304±15.5 |
| Ethanolic 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.

Table 5.10: Effect of ethanolic extracts of *Butea superba* on testosterone level in male rats

| S. No | Group | Testosterone (ng/ml) | |
|-------|-----------------------|----------------------|---------------|
| | | 15 days | 28 days |
| 1 | Control | 1.75 ± 0.28 | 1.78 ± 0.21 |
| 2 | Ethanolic Extract 50 | 1.81 ± 0.24 | 2.16 ± 0.28 |
| 3 | Ethanolic Extract 100 | 2.00 ± 0.26 | 3.81 ± 0.23** |
| 4 | Ethanolic Extract 200 | 2.20 ± 0.21* | 4.20 ± 0.23** |

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. Sertoli 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 Leydig 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.

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REFERENCES

1. Rullyansyah S, Muzakky F, Samlan K, Hanistya R, Maulidiyanti ETS. (2022). Phytochemical screening and evaluation of red ginger extracts on aphrodisiac activity. *Gac Med Caracas* [Internet].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. (2022). Zein-stabilized nanospheres as nanocarriers for boosting the aphrodisiac activity of icariin: Response surface optimization and in vivo assessment. *Pharmaceutics* [Internet]. 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. (2020). Aphrodisiac activity of nutraceuticals. *Int J Res Pharm Sci* [Internet]. ;11(3):3878–88. Available from: <http://dx.doi.org/10.26452/ijrps.v11i3.2572>
6. Singh SK, Jain AP, Tandon M. (2020). Investigations on Citrullus colocynthis seeds for aphrodisiac activity in male albino rats. *Adv Pharm J* [Internet]. 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. (2020). Evaluation of the potential aphrodisiac activity of sesquiterpenoids from roots of *Ferula huber-morathii* Peşmen in male rats. *J Ethnopharmacol* [Internet]. ;257(112868):112868. Available from: <http://dx.doi.org/10.1016/j.jep.2020.112868>
8. Choudhury MRA, Basak M. (2021). Ethno medicinal plants used for aphrodisiac activity in North-East, India. *J Pharm Res Int* [Internet]. 314–22. Available from: <http://dx.doi.org/10.9734/jpri/2021/v33i38b32129>

9. Gupta M, Mondal AK. (2020). Clinical evaluation of aphrodisiac activity of a novel ayurvedic formulation for treatment of male sexual disorders. *Int J Res Med Sci* [Internet]. 8(7):2515. Available from: <http://dx.doi.org/10.18203/2320-6012.ijrms20202888>
10. Vasanttrao US, Biyani KR. (2020). Evaluation of Preclinical Aphrodisiac Activity of *Flueggea leucopyrus* Willd. Leaves. *Curr Bioact Compd* [Internet]. 16(3):335–41. Available from: <http://dx.doi.org/10.2174/1573407214666181105115510>
11. Kadzo L. (2020). Aphrodisiac Activity of Pomegranate (*Punica granatum* L.) fruit extract on the sexual function in rats. *Discov Phytomedicine* [Internet]. 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. Pratik Dhoknea , Renuka Mahajana , Prakash Itankar, Nandini Bhojraj. (2020). 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]. 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 *lepidium 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.

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