

Evaluation of *Litsea glutinosa* bark on Immobilization Stress Induced Sexual Behavior and Fertility of Male Rats.

Pattari Lohitha., I.S. Muchandi., Yogesh.H.S., Shambhu. C.K., V.M.Chandrashekar*.

*Department of Pharmacology, H.S.K College of Pharmacy, Bagalkot -587101, Karnataka, India.

Summary

Physical or emotional stress is a profound disruptive factor to the reproductive function. In males, stress induces suppression of testosterone secretion, spermatogenesis and libido. On the other hand, chronic stress by immobilization has been reported to produce an increase in circulating levels of adrenocorticotrophin, as well as general inhibitory effect on pituitary-gonadal function, in males, chronic stress induces low circulating levels of testosterone, prolactin and follicle stimulating hormone. *Litsea glutinosa* bark extracts was evaluated for aphrodisiac as well as infertility treatment activity by studying sexual behavior parameters, number of mounts, ejaculation latency, intromission interval, number of ejaculations, histopathological studies, sperm density, sperm motility for normal as well as immobilization stress induced male wistar albino rats. 300 mg/kg ethanolic and 500 mg/kg aqueous extract possess significant ($P<0.05$) aphrodisiac activity, as compared to normal animals. Significant increase number of mounts ($P<0.001$), ejaculation latency ($P<0.001$), intromission interval ($P<0.001$), number of ejaculation ($P<0.001$) s, and decreased latency of first mount ($P<0.001$) as comparison to control animals .This study provides evidence for significant aphrodisiac and possible male anti-infertility activity with improved testicular performance.

Key words: *Litsea glutinosa* bark, testosterone, male anti-infertility, immobilization stress.

***Corresponding author:**

Chandrashekhar.V.M
Department of Pharmacology
H.S.K College College of Pharmacy,
Bagalkot- 587101, Karnataka, India
Tel: 91-9880298342,
E-Mail: chandupharm@yahoo.com

Introduction

The endocrine response to stress is not limited to activation of the hypothalamo-hypophyseal-adrenocortical system, but involves the hypothalamo-hypophyseal-gonadal system and other neuroendocrine axis. Physical or emotional stress is a profound disruptive factor to the reproductive function. In males, stress induces suppression of testosterone secretion, spermatogenesis and libido. On the other hand, chronic stress by immobilization, intermittent electric foot shocks, surgery or crowding has been reported to produce an increase in circulating levels of adrenocorticotrophin (ACTH), as well as general inhibitory effect on pituitary-gonadal function, both in males and females. In males, chronic stress induces low circulating levels of testosterone (T), prolactin and follicle stimulating hormone (FSH).²

There are evidences that environmental factors, whether chemical, physical or emotional may adversely affect the testicular functions. Immobilization (restraint) stress has been commonly used by other workers as stress inducers in rats. In the present project we have studied the effect of immobilization stress, which acts as physical as well as psychological stressor, on the testis of albino rats¹.

Synthetic drugs like sildenafil citrate, vardenafil and tadalafil citrate are used for erectile dysfunction but these drugs have fatal side effects like sudden hypotension, hypersensitivity reaction, myalgia, abnormal vision, etc. and infertility³.

The present study with *Litsea glutinosa* bark selected on the basis of traditional aphrodisiac claim. The bark of *Litsea glutinosa*, which according to kirtikar and Basu, “is one of the most popular of native drugs”, is considered to be capable of relieving pain, arousing sexual power and also, producing a soothing effect on the body, good for the stomach are considered to be mildly astringent, other uses of the bark include treatment of diarrhea and dysentery⁴.

Materials and Methods:

Materials

REMI research centrifuge (R-24), Borosil soxhlet extractor, solvent evaporator, Research microscope (Metzer), Afcoset Digital balance (E-R-180 A), Mini lyotrap (LTE Scientific Ltd, Great Britain) and Handy cam (Sony). Testosterone injections (250mg/ml) [Sustanon], Organon (India) Ltd, Kolkata BNO: 252301 were purchased form local market. Sildenafil citrate was obtained as gift sample from Cipla pharmaceuticals, Mumbai, India. Progesterone injections (50 mg/ml), [Susten 200], Unimed technologies Ltd Halol, Gujarat BNO: 70654 were purchased from

local market. Estradiole valerate injection (10mg/ml), [Progynon Depote], Company - Cadila health care Ltd Ponda, Goa, India, BNO: GH 1029 was purchased from local market. Ketamin injection (50mg)-Neon Laboratories Ltd. Mumbai, India, was purchased from local market. Tween-80 (S, d, fine Chem. Ltd, Mumbai.), Arachis oil, and Olive oil, were purchased from local market. All chemicals and solvents used in this study were of AR grade.

Plant material

Litsea glutinosa bark was collected in the month of August- September from local area of Harapanahalli; Karnataka herbarium was prepared by processing the plant for 20 days. The identity of *Litsea glutinosa* was confirmed at Basaveshwara Science College by botanist Prof. S.A. Kappali Bagalkot, Karnataka voucher Specimen (B.sc/Bot/07/08-09) was deposited in herbarium of Basaveshwar science college Bagalkot. 10 Kg of bark was crushed to coarse powder and passed through sieve # 44. The sieved powder was stored in air tight, high density poly ethylene containers before extraction.

Preparation of plant extract

The powdered material of *Litsea glutinosa* bark was extracted separately by continuous hot extraction process using Soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, ethanol, to finally chloroform: water after extraction the extracts were subjected to Lyotrap dryer, preserved in aseptic condition the percent yields were calculated based on the total amount of dried powder and dose were selected based on acute toxicity study. The dried extracts reconstituted in suspension in 5% Tween 80 and administered orally tared vessels the dried extracts were subjected to various chemical tests to detect the presence of different phyto constituents like alkaloids, tests for glycosides, sterols, starch mucilage, proteins, cardiac glycosides, saponins, tannins and flavonoids.⁵⁻⁶

Animals

Healthy male Wister albino rats of weight 150-200g⁷, and female Wister albino rats of weight 140-200 g were used in this study. Female Swiss albino mice were used for acute toxicity studies. The animals were kept in standard conditions, were fed with standard pellets (Amruth, Sangali) and *ad-libitum* water. The experimental procedure was conducted as per guidelines of the Institutional animals ethics committee (IAEC, Clearance/2007/1-8), H.S.K. College of Pharmacy, Bagalkot, Karnataka.

Methods:***Acute toxicity***

Acute oral toxicity study was performed as per OECD-423 Guidelines. Female Swiss albino mice (20-25gm) were randomly distributed to 6 groups (n=6) the animals were fasted overnight and drug was administered orally at the dose of 100, 200,400,800,1600 and 3200 mg/kg the animals were closely observed for 24 hr for an toxic symptom and 72hr for motility rate.

Induction of Immobilization stress

The animals were subjected to IMB stress by Plexiglas cylinder (5 cm diameter and 16 cm large) for 6 h a day during light period started from 8 am each day for 20 consecutive days. Water and food were withdrawn during stress period.¹The Wister Albino rats were divided into ten groups of six animals each, in which three groups were for aqueous extract and other three groups received ethanolic extract in a dose of 100, 300 and 500 mg/kg respectively. First group was served as normal without any treatment. Second group served as control which received vehicle (5 ml/kg) of 5% Tween-80 p.o, Third group served as standard received Testosterone 15mg/kg, i.m⁸, Fourth group served standard Sildenafil citrate 0.7 mg/kg i.p⁹. All the treatments were given for 20 consecutive days¹.

A total number of 80 male Wister albino rats weighing 150-200g were taken and randomly divided into 10 groups of 6 in each group. Group first as normal not receive any treatment. Group second served as control was received vehicle (5 ml/kg) 5% Tween-80 and stress by immobilization (IMB). Group third was standard and was received testosterone 15 mg/kg per rat i.m, and stress by IMB, Fourth group served for Sildenafil citrate 0.7 mg/kg i.p and group fifth, sixth and seventh received aqueous extracts 100 mg/kg, 300 mg/kg and 500 mg/kg respectively and stress by IMB, the group eighth, ninth and tenth, and received ethanolic extracts 100 mg/kg, 300 mg/kg, and 500mg/kg respectively and stress by IMB. All extracts were given by orally gastric gavages.

Evaluation of stress induced aphrodisiac activity, these rats were individually placed in cages 3 h following the administration of treatment and were given 10 min adaptation period. A receptive female rat was taken for each male. To make receptive female, that had been brought into oestrus (oestradiole benzoate 12 µg in olive oil) injected subcutaneously 56 hours prior to plus progesterone 0.5 mg in olive oil injected subcutaneously 8 hours prior to pairing) was placed in the cage before pairing¹². The following parameters of sexual behavior were monitored 20 min after pairing, under dim light and video recording was done by using Sony Handy cam. Latency of first mount, number of mounts, latency of first intromission, inter intromission interval, number of

intromission, latency of first ejaculation, latency of second ejaculation, average ejaculation latency and number of ejaculations was recorded¹².

Sperm density

Spermatozoa were collected by flushing the vasdeferens and epididymis in 2.0ml of normal saline, draw the semen in the WBC pipette up to 0.5 marks, and diluted to 11 mark by using 4 % sodium bicarbonate in 1 % phenol solution. That makes a dilution of 1 in 20. Thoroughly shake the mixture, discard the first few drops and then add 2 to 3 drops on a neubauer's counting chamber and observe under light microscope. Sperm count/ml was calculated as given below¹³. Total number of sperms / ml plasma = N x 50000, Where N is the total sperm count observed in outer four square of WBC chamber.

Sperm motility

The relative proportions of the normal and abnormal sperms were calculated by smear preparation according to the method of Bauer *et al.*, (1974). Equal volume of Cauda epididymal plasma and 5 % sodium bicarbonate were taken in a centrifuge tube, mixed well and centrifuged for 5 minutes at 4000 g. The supernatant was discarded and to the precipitate 5 ml of normal saline was added, mixed well and centrifuged again. The procedure was repeated 2 to 3 times and a clear precipitate was obtained. To the final precipitate few drops of normal saline were added, mixed thoroughly and a smear was prepared on a clean slide. The smear was dried at room temperature, fixed by heating it over the flame for two to three seconds. Then the smear was flushed with 95 % alcohol, drained and dried. It was stained in Ziehl Neelson's Carbol Fuchsin diluted with equal volume of 95% alcohol for 3 minutes and counter stained with 1:3 (v/v) aqueous solution of Loeffler's methylene blue for 2 minutes (Gurr, 1956). After staining, the smear was rinsed in water and dried in air. The abnormal sperms included categories like double tailed, detached head, detached tail, mid piece bending and irregular head. The relative proportions of the normal and abnormal sperms were from the smear and are expressed in terms of percentage¹⁴.

Histopathological studies of testis

Two left testis of each group were excised and rinsed in 0.9% saline blotted dry of saline and excess blood. They were fixed in 12 % formalin for 24 hr. The tissues, after fixation, were washed in water to remove excess fixative. Washed tissues were then dehydrated through a graded series of ethyl alcohol, cleared with Chloroform and embedded in paraffin wax. Sections were cut at 3 µm with microtone blade, and mounted on clean glass slide.

The sections were routinely stained with haematoxyllin and eosin. The stained slides were observed (40X) in research microscope and photographed.

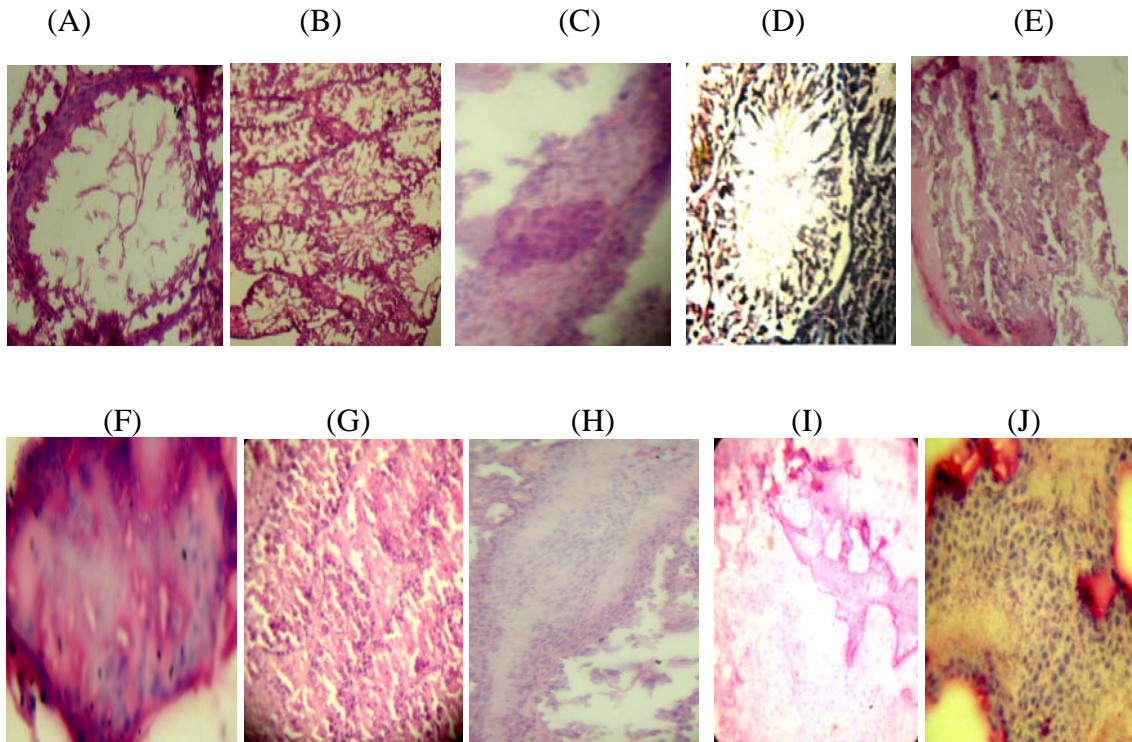


Figure (A) shows normal spermatogenesis and histological structure of the seminiferous tubules and intertubular spaces. Figure (B) shows decrease in the spermatogonial cells normal architecture is damaged .Figure (C) we can observe increase in the spermatogonial cells are observed normal architecture get retained. Similarly from figure (D) we can observe increase in the number of spermatogonia cells, and normal architecture of cell. In figure (E) the normal architecture is damaged but more number of spermatogonial cells can be visible and figure (F) we can view normal architecture of cell and also increase in the spermatocytes number. The figure (G) we can see the damage of normal architecture of the cell and increase in the intra cellular space of seminiferous tubules and the figure (H) we come to reveal that there is increase in the spermatocytes number. Figure (I) there is increase in the spermatogonial cells and normal architecture is damaged and intra cellular space is less and the figure (J) there is swelling in the spermatogonial cells intra cellular space is more.

Statistical analysis

Data collected in the study are expressed as the mean ± standard error of mean (S.E.M.) and statistical analysis was carried out by using one-way analysis of variance (ANOVA) method. P value of less than 0.05 was considered to be statistically significant.

Results

Acute oral toxicity:

The aqueous and ethanolic extracts of *Litsea glutinosa* does not produced any mortality up to 3200mg/kg body weight further dosing was not performed to estimate the LD₅₀ (Lethal dose) value. According to the OECD guidelines for the acute toxicity, an LD₅₀ dose 2000mg/kg body weight and above is categorized as unclassified and hence the drug is found to be safe.

Effect of Litsea glutinosa on Stress modulated sexual behavior in male rats:

From table I and II we revealed that significant decrease in latency of first mount with aqueous extract 500 mg/kg 176 ± 31 (P<0.01), with ethanolic extract 500 mg/kg 233 ± 27.9 (P<0.05) and total latency ejaculation 17 ± 1.3 (P<0.01) with aqueous extract 100 mg/kg.

Sperm density

The sperm density is significantly declined to 6.9 ± 0.6 in stressed rat when compared to normal group 7.57 ± 2.17 . And the aqueous extracts with moderate and higher dose will enhance the sperm count to 16.3 ± 2.4 and 18.4 ± 0.5 with (P value <0.05) with respect to following doses 300 mg/kg and 500 mg/kg. In table - VI ethanolic extract of 500 mg/kg 17.6 ± 3.1 (P<0.05) shown comparative increase in sperm count and almost equal as that of standard drugs Sildenafil citrate and Testosterone.

Sperm motility

The sperm movement also decreased in stressed rats by 63.5 ± 4.5 , when compared with normal group which had shown 86 ± 5 , Standard testosterone 15 mg/kg s.c is 88.5 ± 2.5 (P<0.01) , Sildenafil citrate 0.7 mg/kg i.p 72.5 ± 3.5 (P<0.05) , Here in table -V the aqueous extract 500 mg/kg and 300 mg/kg shown 64 ± 5 (P<0.05) and 88.5 ± 2.5 (P<0.05) , while ethanolic extract shown significant with dose of 500 mg/kg 84.5 ± 6.5 (P<0.05).

Table-I. Effect of *Litsea glutinosa* bark aqueous extract on stress modulated sexual behavior of male rats.

| Treatment | Latency of first mount (sec) | No. of mounts | Latency of first intromission (sec) | Inter intromission interval (sec) | Total Latency of Inter intromission interval (sec) | Latency of first ejaculation (sec) | Latency of second ejaculation (sec) | Total Latency of ejaculation (sec) | Ave. ejaculation latency (sec) | No. of ejaculation |
|---|------------------------------|---------------|-------------------------------------|-----------------------------------|--|------------------------------------|-------------------------------------|------------------------------------|--------------------------------|--------------------|
| Normal | 355 ±29.9 | 5.66±0.76 | 4.4 ± 0.33 | 32.5±2.83 | 361± 31.6 | 321 ± 0.59 | 3.2 ± 0.2 | 24.6 ± 1.1 | 3.2 ± 0.31 | 6.5± 1.5 |
| Vehicle tween-80 5% (5ml/kg po) in stressed rat | 257 ± 18.9* | 7.5 ± 1.2 | 4.1±0.3 | 25.8 ± 4.9 | 296.7 ± 49 | 3.5±0.38 | 3 ± 0.25 | 17.8± 2* | 3.3 ±0.31 | 6.1± 1.2 |
| Testosterone (15mg/kg,i.m) in stressed rat | 154 ± 15*** | 11.1± 1.9* | 5.2± 0.37 | 36 ± 5.3 | 213 ± 10** | 4.7± 0.42 | 3.63±0.31 | 29 ± 1.6* | 4.1 ± 0.35 | 13 ±1.3** |
| Sildenafil citrate (0.7mg/kg i.p) in stressed rat | 171±14*** | 9.8± 1.3* | 4.68 ±0.66 | 32.6± 4.6 | 245 ± 27.1* | 4.6 ± 0.25 | 3.4 ± 0.22 | 24.3 ± 1.2 | 4.02±0.17 | 10.5±2.55 |
| Aqueous Extract (100mg/kg p.o) | 379 ± 58.6 | 5.66 ±1.8 | 3.9 ± 0.51 | 24.3±5.84 | 416±41.5 | 2.1±0.32 | 2.25±0.2* | 17 ±1.3** | 2.1±0.23* | 6.16±1.3 |
| Aqueous Extract (300mg/kg p.o) | 232.3±36.4* | 7.5±2.84 | 3.9±0.66 | 45.8±4.3 | 379.7±51.5 | 3.2±0.7 | 2.4± 0.47 | 19.6±2.2 | 2.8 ± 0.58 | 6.5±0.76 |
| Aqueous extract (500mg/kg p.o) | 176 ± 31*** | 9.33 ± 1.7 | 4.43±0.82 | 33.6±2.44 | 307.2±59.8 | 3.83±0.58 | 2.9±0.53 | 21.3±2.2 | 3.36±0.54 | 7±1.34 |

Values are expressed as mean ± SEM. and analyzed by One way Analysis of variance (ANOVA) followed by *Dunnett's t* test n=8, *P< 0.05, **P<0.01, ***P<0.001.

Table-2. Effect of *Litsea glutinosa* Ethanolic extract on stress modulated sexual behaviour on male rat

| Treatment | Latency of first mount (sec) | No. of mounts | Latency of first intromission (sec) | Inter intromission interval (sec) | Total Latency of Inter intromission interval (sec) | Latency of First ejaculation (sec) | Latency of second ejaculation (sec) | Total Latency of ejaculation (sec) | Ave. ejaculation latency (sec) | No. of ejaculation |
|---|------------------------------|---------------|-------------------------------------|-----------------------------------|--|------------------------------------|-------------------------------------|------------------------------------|--------------------------------|--------------------|
| Normal | 355 ± 29.9 | 5.66 ± 0.76 | 4.4 ± 0.33 | 32.5 ± 2.83 | 361 ± 31.6 | 321 ± 0.59 | 3.2 ± 0.2 | 24.6 ± 1.1 | 3.2 ± 0.31 | 6.5 ± 1.5 |
| Vehicle tween-80 5% (5ml/kg p.o) in stressed rat | 257 ± 18.9* | 7.5 ± 1.2 | 4.1 ± 0.3 | 25.8 ± 4.9 | 296.7 ± 49 | 3.5 ± 0.38 | 3 ± 0.25 | 17.8 ± 2* | 3.3 ± 0.31 | 6.1 ± 1.2 |
| Testosterone (15mg/kg i.m) in stressed rat | 154 ± 15*** | 11.1 ± 1.9* | 5.2 ± 0.37 | 36 ± 5.3 | 213 ± 10** | 4.7 ± 0.42 | 3.63 ± 0.31 | 29 ± 1.6* | 4.1 ± 0.35 | 13 ± 1.3** |
| Sildenafil citrate (0.7mg/kg i.p) in stressed rat | 171 ± 14*** | 9.8 ± 1.3* | 4.68 ± 0.66 | 32.6 ± 4.6 | 245 ± 27.1* | 4.6 ± 0.25 | 3.4 ± 0.22 | 24.3 ± 1.2 | 4.02 ± 0.17 | 10.5 ± 2.55 |
| Ethanolic extract (100mg/kg p.o) | 404 ± 63.8 | 5.66 ± 2.0 | 3.5 ± 0.7 | 28.1 ± 5.1 | 387 ± 37.7 | 2.6 ± 0.47 | 2.5 ± 0.36 | 18.5 ± 2.6 | 2.5 ± 0.41 | 4.83 ± 1.5 |
| Ethanolic extract (300mg/kg p.o) | 407.5 ± 31.5 | 6.5 ± 2.5 | 2.9 ± 0.51 | 29 ± 4.2 | 363 ± 57.7 | 2.63 ± 0.29 | 2.63 ± 0.22 | 19 ± 2.03* | 2.63 ± 0.25 | 7.16 ± 2.08 |
| Ethanolic extract (500mg/kg p.o) | 233 ± 27.9* | 8.16 ± 2.7 | 4.5 ± 0.9 | 32.6 ± 5.8 | 235 ± 18** | 4 ± 0.45 | 3.2 ± 0.37 | 24.6 ± 1.5 | 3.6 ± 0.36 | 9.3 ± 2.3 |

Values are expressed as mean ± SEM. and analyzed by One way Analysis of variance (ANOVA) followed by Dunnett's 't' test, n=8, *P< 0.05, **P<0.01, ***P<0.001

Discussion

The results of the present study, indicates that both aqueous and ethanolic extract of *Litsea glutinosa* bark possess significant aphrodisiac property as well as their treatment could be useful to overcome stress induced male sexual dysfunction. However, more promising and significant effects were observed in ethanolic treated group of male rats, as observed in improved sexual performance decreased latency with increased number of ejaculations supported by histopathology results.

The aphrodisiac activity obtained from the present study with sildenafil citrate and testosterone is similar to the results reported in earlier studies¹⁵. The fourteen days treatment with their drugs increased number of mounts, number of ejaculations with decrease intromission interval. The ethanolic extract treatments have shown no considerable and significant activity. However in aqueous extract treatment group the potent male aphrodisiac activity was observed linear dose dependently. The fourteen days treatment, significantly increased number of mounts, number of ejaculations, ejaculation latency, inter intromission interval, and also latency of first intromission indicates that its possible beneficial effects in coping up premature ejaculation, reduced amount of time record for the sexual stimulation and also tendency to reduce post ejaculatory intervals with increased copulatory efficiency. The saponins present in the aqueous and ethanolic extract of this plant might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the level of leutinizing hormone (LH). This LH released normally by the pituitary gland helps to maintain testosterone levels: as LH increases so does the testosterone. The increase in testosterone seemed to have translated into the male sexual competence observed in this study suggests that the aphrodisiac action may be mediated through a change in the blood testosterone level.¹⁶

Immobilization stress causes marked suppression of spermatogenesis. The reason behind it is that restraint is a potent stimulus, which induces depression of hypothalamus-pituitary-axis mediated by activated hypothalamus-pituitary-adrenal –axis, resulting in fall in plasma, LH and testosterone levels. Testosterone and FSH act directly upon germinal epithelium and are required for spermatogenesis¹⁷.

In the present study there is a decrease in the level lipid peroxidation, glutathione and superoxide dismutase (SOD) for all doses of both aqueous and ethanolic extracts. Similarly, increase in the levels of catalase and total thioles, thus the present study has shown antioxidant property and protective mechanism for *Litsea glutinosa*.

Conclusion

In the present study we come to conclude that the plant *Litsea glutinosa* is beneficial as according to traditional claims will be worth full for further human use. From the histopathological studies of stress induced rat testis the 300 mg/kg of both aqueous and ethanolic extracts shows significant effects in recovering the tissues to normal also sperm count, sperm motility was increased and latency of first mount is decreased total latency of intromission also increased is observed.

Acknowledgment

We thankful to, the principal of H.S.K College of pharmacy, Bagalkot, for providing necessary facilities to carryout the research project.

Table-3. Effect of *Litsea glutinosa* bark aqueous extract on sperm density and sperm motility in stress modulated male rats.

| Treatment | Normal | Control (Tween -80) | Testosterone (15mg/kg, i.m) | Sildenafil citrate (0.7mg/kg i.p) | Aqueous extract (100 mg/kg p.o). | Aqueous extract (300mg/kg p.o). | Aqueous extract (500mg/kg p.o). |
|---|-----------|------------------------|--------------------------------|---|---|--|--|
| Sperm density in (Million/ml) | 7.57±2.17 | 6.9 ± 0.6 | 19 ± 3.2** | 18± 0.1* | 8.9±0.4 | 16.3 ± 2.4* | 18.4 ± 0.5 * |
| Percentage Sperm motility in (%) | 86 ± 5 | 63.5 ± 4.5 | 88.5 ± 2.5** | 72.5± 3.5* | 59.5±1.5 | 64 ± 5* | 88.5 ± 2.5* |

Values are mean ± SEM. The results obtained were analyzed by one way of variance (ANOVA) followed by Dunnet's 't'. n=6, * P< 0.05, ** P<0.01, *** P<0.001.

Table-4. Effect of *Litsea glutinosa* bark ethanolic extract on sperm density and sperm motility in stress modulated male rats.

| Treatment | Normal (Control) | Control (Tween-80) | Testosterone (15mg/kg,im) | Sildenafil citrate (0.7mg/kgIp) | Ethanolic Extract (100mg/kg po) | Ethanolic Extract (300mg/kgpo) | Ethanolic Extract (500mg/kg po) |
|-------------------------------------|---------------------|-----------------------|------------------------------|------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|
| Sperm density (Million/ml) | 7.57± 2.17 | 6.9 ± 0.6 | 19 ± 3.2 ** | 18 ± 0.1** | 12.05 ±0.55 | 12.2 ±1.6 | 17.6±3.1* |
| Percentage Sperm motility (%) | 86± 5.55 | 63.5 ± 4.5 | 88.5 ± 2.5** | 72.5± 3.5* | 58.5± 0.5 | 71 ± 4 | 84.5±6.5* |

Values are expressed as mean ± SEM. The results obtained were analyzed by One way ANOVA followed by Dunnet's 't' test, n=6, * P< 0.05, ** P<0.01, *** P<0.001.

References

- 1) Rai, Jyoti; pandey, S.N, Srivastava, R.K. Effect of immobilization effect of stress on Spermatogenesis of Albino Rats. *J Anat. Soc. India*. **52 (1)** 55- 57 (2003)
- 2) Socorro Retana Marquez, Emilio Dominguez Salazar and Javier Velazquez Moctezuma. Effect of acute and chronic stress on masculine sexual behavior in the rat. *Psychoneuroendocrinology*, **Vol.21**, NO.1. PP.39-50, 1996
- 3) Kasper DL, Fauci AS, Longa DL, Braunwald E, Hauser SL, Jameson JL, Harrison's *Principles of internal medicine*. 16th ed. New york Mc Graw –Hill, 2005 PNO: 272-273
- 4) Bikash Rath, VASUNDARA, Medicinal contributions from certain species of *Litsea* P No 1-31
- 5) Dr. C.K. Kokate., Practical pharmacognosy, Preliminary phytochemical screening, Chapter 6 PNo: 106- 111.
- 6) Practical pharmacognosy, *techniques and experiments*, by Khandelwal K.R. Chapter 40, P.NO: 149-153.
- 7) Malti Singh., Natwar R., Kalla, Sankar N. Sanyal., Effect of monensin, a Na⁺- specific carboxylic ionophore on the oxidative defense system in rat testis. *Pharmacological reports* 2007, **59**, 456-461.
- 8) Hooi Hoon ANG, Hung Seong CHEANG, Ahmad Pauzi Md. YUSOF., Effect of eurycoma logifolia jack (Tongkatali) on the initiation of sexual performance of inexperienced Castrated male rats. *Exp. Anim.***49 (1)**, 35-38, 2000.
- 9) Madiha AH Hassan., Amal F Ketat Sildenafil citrate increases myocardial cGMP content in rat heart, decreases its hypertrophic response to iso proterenol and decreases Myocardial leak of creatinine kinase and trophin T. *BMC Pharmacology* 2005,
- 10) University of Plymouth Dept. of psychology, salmon study materials on-line, PSY128 Lecture support material, Hormones and sexual behavior. www.indopedia.org/environment
- 11) OECD 423 guidelines for testing chemicals.
- 12) Ratnasooria, Dharmasiri MG. Effect of Terminalia Catappa seeds on sexual behavior & fertility of male rats. *Asian J Androl* 2000;2:213-19.
- 13) Jain AK, Manual practical physiology for MBBS, Arya publications Sirmour, Semen analysis sperm count and motility 2003;1:176-77.
- 14) Bauer JD, Ackerman PG, Toro G, Clinical Laboratory Methods. St. Louis Missouri, USA: Mosby Co, 1974.
- 15) Boolell M., Gepi-Attee S., Gingell. JC., Allen MJ., Sildenafil, a novel effective oral therapy for male erectile dysfunction. *Br J Urol* 1996;78:257-261.
- 16) Yakubu M. T., Akanji M. A., Oladij A. T. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. *Asian J Androl* 2005; **7 (4)**:399-404
- 17) Rai Jyoti., Pondey S.N., Srivastava R.K. Effect of immobilization stress on spermatogenesis of albino rats: *J anot. Soc.India* **52 (1)** 55-57 (2003).