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Research Article

Citrullus lanatus (Thunb) Improves Antidepressant-Induced Impaired Fertility in Depressed Male Mouse Model

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ABSTRACT

Synthetic antidepressants are used in the treatment of mental illness. However, due to their side effects, people are orienting towards the herbal product as an alternative therapy. The present study, therefore, explores the role of *Citrullus lanatus* (CL) seed extract in evaluating the fluoxetine-induced impaired sperm functions and fertility of depressed mice. Adult Swiss strain male mice were separated into six groups. Every group contains six animals. Group I received vehicle-treated negative control while groups II, III, IV, V and VI were administered reserpine (RES) at a dose of 0.75 mg/kgBW/day, for 14 days to induce depression, fluoxetine (FLX) at the dose of 40 mg/kgBW/day in depressed mice (positive control) for 28 days, CL seed extract (300 mg/kgBW/day) only, in normal mice, and co-administrations of FLX (40 mg/kgBW/day) with low and high doses of seed extract of CL (150 and 300 mg/kg BW/day), respectively, in the depressed mice for the same duration. Mice of Gr. II and Gr. IV did not alter the epididymal sperm parameters and fertility. However, significant alterations were observed in such parameters in the mice of Gr. III. Partial to complete restorations were noticed in such parameters in Gr. V and VI, as compared to the positive control of Gr. III. The findings suggest that CL seed extract may emerge as a potential herbal remedy for impaired fertility, which could be of therapeutic drug in the future.

Introduction

Depression is a mental health problem that affects approximately 280 million people worldwide.[1] Antidepressant drugs are frequently used to treat various health issues, including panic disorders, anxiety, and obsessive-compulsive disorder. However, these medications are negative effects on various biological systems, including the circulatory,[2] nervous,[3] gastrointestinal,[4] and reproductive systems.[5,6] The negative impact of these drugs on reproduction has become a significant concern in light of the increasing prevalence of male infertility by causing alterations in sperm production and their function and erection.[7-9] Fluoxetine (FLX) is the primary antidepressant employed in the management of depression. Intervention of FLX in spermatogenic activity along with other testicular functions has been reported in the depressed mouse.[10,11] Different doses of fluoxetine treatment in the rat cause impaired count, motility and morphology of the spermatozoa.[12] In another study, a virgin female rat impregnated with an FLX-treated male rat showed marked impairment in fertility indicated by fewer pregnancies, a lower number of implantations, and viable fetuses.[13]

Herbal therapy is used widely across the world for its effectiveness with least or no side effects, which has therefore propelled scientific investigation toward the fertility-boosting properties of several plants. Today, extracts, fractions, or molecules derived from different parts of plants have emerged as a natural and well-established medicine for infertility therapy as well as for fertility regulation.[14-16] The Traditional Indian System of Medicine claims a vast number of medicinal herbs that are used in the treatment of impaired fertility, such as *Bacopa monnieri*,[17,18] *Piper longum*,[19] *Tinospora*

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cardifolia,[20] *Zingiber officinale*,[21] *Withania somnifera*,[22] and *Citrullus lanatus*.[23] Among them, *C. lanatus* (common name: watermelon) has been regarded as a novel source of herbal medication for infertility therapy. This flowering plant belongs to a member of the Cucurbitaceae family and possesses a variety of pharmacological properties.[24] Steroidal phytochemicals such as flavonoids, saponin, citrulline, and arginine present in *C. lanatus* (CL) have shown tremendous effects in the sexual performance and management of infertility conditions.[24] Among flavonoids, vitexin is the most abundant bioactive constituent present in its extract, bearing antidepressant as well as aphrodisiac properties.[25,26] The CL seed extract has been reported to improve sperm function and alter fertility in both male and female rats.[27-29] The extract of CL pulp has shown ameliorative effects against monosodium glutamate-induced alterations in sperm motility, viability, and count, as well as altered sperm morphology in the rat. [30,31] Further, the beneficial role of CL rind methanolic extract on altered semen parameters and reproductive hormones against nicotine,[32] phenyl hydrazine,[33] and lead acetate[34]-induced toxicity has been reported in the rat. Another study suggested that fruit extract of CL provides protection to the sperm cells against arsenic-induced oxidative stress in the rat.[35] However, fertility-boosting properties of CL against any antidepressant have not been reported so far. This study focuses on the efficacy of CL seed extract on FLX-induced altered fertility of the depressed mouse.

Materials and Methods

Animals

Swiss strain adult mice (8–12 weeks old; 24–26 g) were purchased to conduct the experimental research. Animals were kept in lab cages with bedded rice husk and provided with pelleted food and water, *ad libitum* with 12 hours of light and 12 hours of dark cycles at 25 ± 2°C. The Animal Ethical Committee, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India, approved the experimental protocol (BHU/DOZ/IAEC/2021-2022/029, February 15, 2022).

Compounds

Reserpine ($C_{33}H_{40}N_2O_9$) and fluoxetine (40 mg) were obtained from Otto Chemie Private Limited, Mumbai, India, and Intas Pharmaceutical Private Limited, Gujrat, India. The *C. lanatus* seeds were extracted by using the method of Adnaik *et al*.[36] The dosage of *C. lanatus* was standardized based on its therapeutic effectiveness in mitigating fluoxetine-induced reproductive changes in depressed mice. Prof. N. K. Dubey, Department of Botany, Banaras Hindu University, verified the authenticity of *C. lanatus* seeds (Voucher specimen no. Cucurbita. 2019/01) purchased from the local market in Varanasi, Uttar Pradesh, India for this study.

Extraction of Plant Materials

Cold maceration extraction was performed on the air-dried, coarsely-powdered *C. lanatus* seeds using 70% alcohol for 72 hours. The liquid extract was filtered and transferred to the rotatory evaporator for complete evaporation. After evaporation, the remaining extract was kept in the refrigerator at 4°C for conducting experiments.

Experimental Design and Dose Administration

Swiss strain adult male mice 36 were separated into six groups. Every group contains six animals. Group I received vehicle (corn oil)-treated negative control while groups II, III, IV, V and VI were treated with reserpine (RES) at the dose of 0.75 mg/kgBW/day, for 14 days to induce depression, fluoxetine (FLX) at a dose of 40 mg/kgBW/day in depressed mice (positive control) for 28 days, CL seed extract (300 mg/kgBW/day) only, in normal mice, and co-administrations of FLX (40 mg/kgBW/day) with low and high doses of seed extract of CL (150 and 300 mg/kgBW/day), respectively, in the depressed mice for the same duration. We administered all the treatments orally.

Animal Sacrifice and Collection of Organs

In 24 hours after the last day of treatment, all male mice were euthanized *via* cervical dislocation to collect the cauda epididymides for sperm analysis. After mating ability and fertility test, females were sacrificed to determine the quantal pregnancy, fertility index, and pre-and post-implantation losses.

Epididymal Sperm Assessment

The epididymal sperm assessment was done by adopting the method of the WHO Laboratory Manual.[37] Cauda epididymidis of each mouse from every group was chopped separately in the normal saline to analyze the motility, viability and count at room temperature. The sperm abnormalities were observed by examining a smear on a cleaned microscopic glass slide stained with eosin-nigrosin under a light microscope at 40X and applying the method of Wyrobek and Bruce[38] and Zaneveld and Polakoski.[39]

Mating Ability and Fertility

This test was performed by placing six males from each group separately with two proestrus virgin females for one night. The capacity of the males to mate with virgin females and their fertility were evaluated based on the appearance of vaginal pugs in cohabited virgin females and the presence of implantation sites in such females, respectively. The ovaries and uteri were removed on the 15th day of gestation in order to count the corpus luteum and implantation sites. The implantation sites were stained blue-black by hemosiderin pigment, which was deposited in a 10% ammonium sulfide solution.[40] The live implants, along with pre-and post-implantation losses, were also noticed. The percentage of libido and fertility index, quantal pregnancy, along pre-and post-implantation losses were calculated by the method of Kumari and Singh.[62]

Statistical Analysis

The libido, fertility, and quantal pregnancy indices were analyzed using the Chi-square test. Mean ± SEM for each group was used to express the live implants and pre-and post-implantation losses. One-way ANOVA followed by Post hoc Dunnett's test was used for analysis. The data analyses were conducted using SPSS software version 17 (IBM, USA) at $p < 0.05$. The mean ± SEM for six animals is used to express the values.

Results

Epididymal Sperm Characteristics

The viability, motility, count and abnormalities remained unaltered in the mice of Gr. II and Gr. IV, as compared to that of Gr. I while such parameters showed significant reductions in the epididymis of the mice of Gr. III, as compared to that of Gr. I and II (Figs 1 and 2). Such altered sperm parameters exhibited gradual restorations in the epididymis of the mice of the Gr. V and Gr. VI, similar to that of Gr. I (Figs 1 and 2).

Libido Index

The libido index of the male mice of Gr. II and Gr. IV was 100%, as compared to that of the Gr. I while it showed a significant reduction to 66.67% in the mice of Gr. III, as compared to Gr. I and II (Table 1). The libido index in the mice of Gr. V and VI were restored to 82.62 and 88%, respectively (Table 1).

Quantal Pregnancy and Fertility Index

The quantal pregnancy and fertility index were 100% in the mice of Gr. II and IV, as compared to Gr. I (Table 1). Marked reductions were noted in the percentage of quantal pregnancy (25%), and fertility index (16.67%) in the mice of Gr. III, as compared to Gr. I and II (Table 1). The quantal pregnancy and fertility index of Gr. V and Gr.VI showed a gradual significant increase from 80% and 55 to 90% and 75%, respectively (Table 1).

Live implants, Pre-and Post-implantation Losses

No marked alterations were noticed in the count of live implants, pre-and post-implantation losses in the mice of Gr. II and Gr. IV, as compared to that of Gr. I. However, a significant decline was noted in such parameters in the mice of Gr. III, as compared to the Gr. I and II (Table 2 and Fig. 3). The mice of Gr. V and Gr. VI showed significant partial to complete restorations in such parameters, similar to that of Gr. I (Table 2 and Fig. 3).

Discussion

The present work focused on the therapeutic effects of CL seed extract on sperm characteristics like motility, viability, count and abnormalities, and mating ability and fertility against fluoxetine (FLX) treatment in depressed male mice.

Unaltered sperm assessment in the depressed mice of Gr. II is consistent findings reported in the rat model

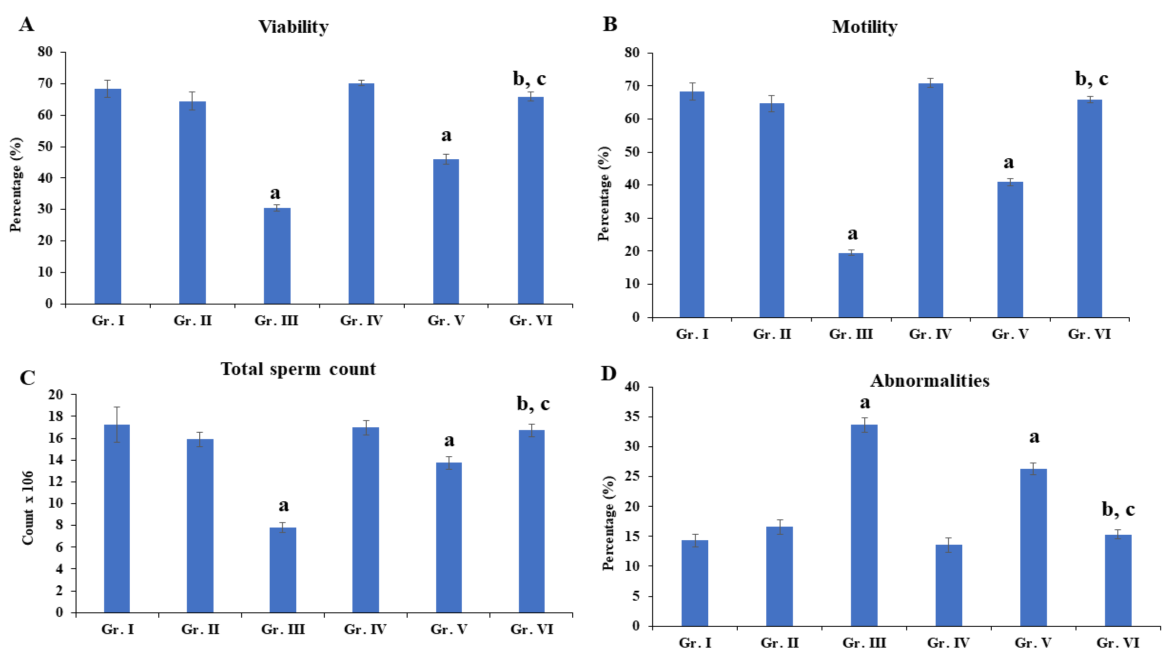

Fig. 1: Showing (A) Sperm viability, (B) Motility, (C) Count, (D) Abnormalities in the cauda epididymidis of control and treated mice. A denotes a significant change from Gr. I and II. B denotes a significant change from Gr. III. C denotes a significant difference from Gr. V at $p < 0.05$. The mean ± SEM for six animals is used to express the values.

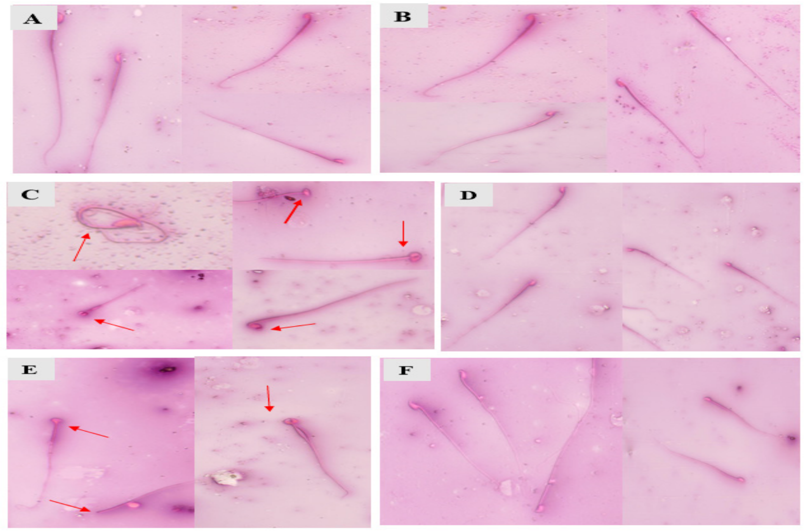

Fig. 2: Spermatozoa obtained from the cauda epididymidis of a control mouse and treated groups. Normal morphological features of spermatozoa with hooked head and straight long tail in the mice of Gr. I (A) and Gr. II (B). The various form of morphological abnormalities in the spermatozoa including amorphous head, hookless head, bi-headed (bicephalic), coiled tail along with normal spermatozoa (red arrows) in the mice of Gr. III (C). The normal morphological features of majority of the spermatozoa were seen in the mice of Gr. IV (D). Partial to complete recovery was noticed in the morphological features of majority of the abnormal spermatozoa in the mice of Gr. V (E) and VI (F)

of depression.[41] Semen analysis is considered *as* an aspect of the assessment of male factor infertility.[42] Sperm motility, viability, count, and abnormalities are the key indices of fertility that are the fair milestone of spermatogenesis and maturation of spermatozoa in the epididymis. The alterations in sperm characteristics may lead to male infertility.[43] Significantly reduced sperm count in the FLX-treated mice of Gr. III is accordant with Sakr *et al.*[8], who found the same result in the normal/intact rat administered with FLX (10 mg/kg/day) for 28 days. Reduced number of sperm is the consequence of suppressed spermatogenic activity.[44-46] FLX-induced inhibited spermatogenic activity has been reported in our earlier study also.[11] Viability is an essential component for spermatozoa quality analysis and successful fertilization.[47] Reproductive function depends on metabolic activities. Therefore, the metabolic status of spermatozoa can predict sperm fertilizing capacity.[48] Significantly reduced viability of epididymal spermatozoa in the FLX-treated mice of Gr. III denotes the disturbance in their metabolic activity. Sperm motility suggests sperm fertility during epididymal transit, indicating proper spermatogenesis and maturation of spermatozoa. Diminution in the epididymal motile spermatozoa in the FLX-induced mice of Gr. III, found in the present study is accordant with a majority of the previous reports.[8,49-51] A significantly increased abnormal spermatozoa percentage in the FLX-treated mice of Gr. III indicates its toxic effects on fertility. Abnormal sperm morphology is not the primary cause of infertility; however, it may be one contributing factor to infertility. Sialic acid is a type of sugar molecule that plays an influential role in sperm function and fertility. Alterations in the epididymal sialic acid may contribute to the abnormal shape and structure of the spermatozoa.[52] Our ongoing study (unpublished data) has also found significantly reduced epididymal sialic acid concentration in the fluoxetine-treated depressed mice. Significantly increased oxidative stress leading to spermatogenic arrest, as reported in our earlier study,[11] might have damaged the sperm DNA, proteins, and lipids,[53] leading to reduced count, motility, viability and increased abnormalities of spermatozoa.

Table 1: Showing the libido index, quantal pregnancy and fertility index of the control and treated groups. * denotes a significant difference from Gr. I and II. # denotes significant change from Gr. III. **Δ** denotes significant change from Gr. V at $p < 0.05$.

Groups	*Libido index (%)*	*Quantal pregnancy (%)*	*Fertility index (%)*
Gr. I	100	100	100
Gr. II	100	100	100
Gr. III	66.67*	25*	16.667*
Gr. IV	100	100	100
Gr. V	82.62*	80*	55*
Gr. VI	88.34#, Δ	90#, Δ	75#, Δ

Unaltered sperm parameters in only CL-treated mice of Gr. IV has also been reported in rats following administration of rind and fruit pulp extract of CL.[28,32] Marked restorations in such parameters in the mice of Gr. V and VI may be due to the beneficial effects of CL against fluoxetine treatment, which was reported earlier to restore the functions of spermatozoa due to the

Table 2: Showing the live implants, pre-and post-implantation losses of the control and treated groups. *denotes significant change from Gr. I and II. # denotes significant change from Gr. III. Δ indicates a significant change from Gr. V at $p < 0.05$

Groups	*No. of live implants*	*Pre-implantation loss*	*Post-implantation loss*
Gr. I	8.33 ± 0.284	3.001 ± 0.271	0.665 ± 0.189
Gr. II	7.83 ± 0.385	3.166 ± 0.327	1 ± 0.287
Gr. III	0.75 ± 0.509*	0.329 ± 0.723*	6.67 ± 0.924*
Gr. IV	8 ± 0.409	3.66 ± 0.286	0.584 ± 0.183
Gr. V	7.87 ± 0.528*	1.98 ± 0.416*	0.88 ± 0.138*
Gr. VI	8.166 ± 0.548#,Δ	2.96 ± 0.346#, Δ	1.08 ± 0.229#, Δ

presence of several bioactive phytoconstituents such as lycopene, L-citrulline, vitamin C, glutathione and vitexin. These are effective in neutralizing ROS, protecting sperm from lipid peroxidation and oxidative DNA fragments,[54] and contributing to improving the quality of semen by increasing the sperm count, motility, morphology,[55] and integrity of its membrane, which are essential for successful fertilization. L-citrulline may also have a role in supporting testosterone production by enhancing blood supply and nutrients to the Leydig cells.[56] Another active phytoconstituent, vitexin, also plays an essential role in reducing oxidative stress, thus maintaining testosterone biosynthesis and sperm functions.[57]

The libido index is an important parameter dependent on androgen.[58] Unaltered libido index found in mice of Gr. II reflects the optimal level of testosterone. However, a significantly reduced libido index in the mice of Gr. III shows a significant decrease in the testosterone hormone. Decreased testosterone levels in FLX-treated depressed mice have also been reported in our previous study.[11] Mating behaviors in rats and most animals are closely linked to reproductive physiology, behavioral oestrus, and ovulation timing.[59-61] Significant decreased percentage of quantal pregnancy and fertility index in the mice of Gr. III arise as a consequence of inhibited spermatogenic activity. Such decline has also been reported previously during metronidazole exposure in mice[62], suggesting that the altered sperm quality impairs the fertilizing potential of the spermatozoa.

Unaltered quantal pregnancy and fertility index observed in the mice of Gr. IV has also been reported previously by Watcho *et al.*[63] in the rat by the treatment of *Mondia white*. Restorations in such parameters in the mice of Gr. V and VI are consistent with the alterations noticed in the metronidazole-induced mice treated with *Tribulus terrestris*.[62] According to these authors, improved fertility is the consequence of good quality and quantity of spermatozoa.

The live implant, pre-and post-implantation losses remained unaltered in our study, indicating that depression in the mice of Gr. II does not impair sperm quality. On the other hand, marked reductions in such parameters were noticed in the mice of Gr. III indicates a diminution in sperm quality and altered oxidative stress. Unaltered live implants, pre-and post-implantation losses in the mice of Gr. IV is consistent with the finding of Kumari and Singh[62] in the same animal treated with *T. terrestris*. Restorations in such parameters in the mice of Gr. V and VI have also

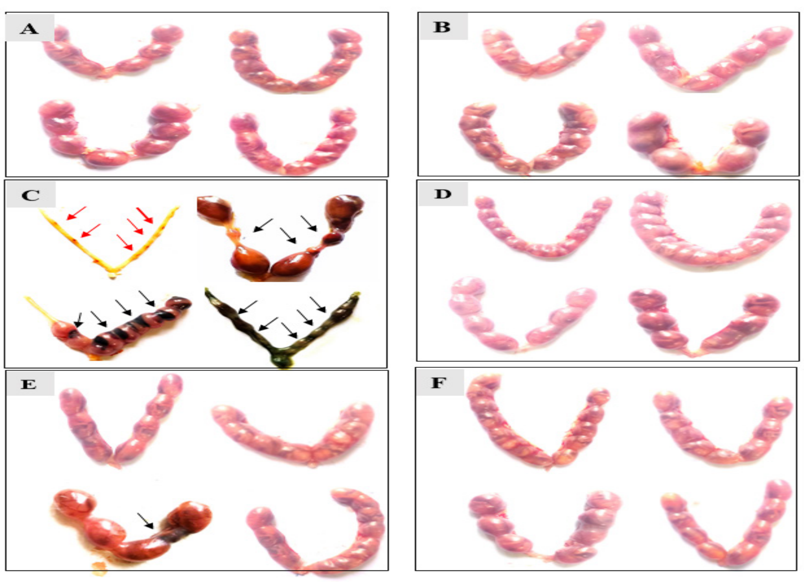

Fig. 3: Uterine implantations of mice of Gr. I (A), Gr. II (B), and Gr III (D). Note the left and right uteri possess a normal range of implanted embryos. Uterine implantations of mice of Gr. III (C). Note the signs of post-implantation loss marked by the low number of implanted embryos in the uterus. Red arrows highlight the dead implant and the resorption sites, marked with black arrows. These sites are identified by scars in the uterine tissue where embryos have not been implanted. Partial to complete recovery was noticed in the implanted embryos in the uterus of the mice of Gr. V (E) and VI (F).

been reported previously by Kumari and Singh[62] in the metronidazole-exposed mice treated with *T. terrestris*. The favorable effects of the CL seed extract on the quantity and quality of spermatozoa may be the cause of the increases in the live implants, along with pre- and post-implantation losses, hence boosting the reproductive potential of the FLX-treated mice. However, the exact mechanism behind the improvement of impaired fertility is not known hence, further studies are to be continued toward the molecular mechanism of action of seeds of CL as fertility enhancers.

Conclusion

The present findings suggest the CL seed extract protects the impaired male fertility in the mice induced by FLX. Hence, this plant may give a clue in the development of a novel drug for the treatment of impaired fertility.

Merits: *Citrullus lanatus* possesses several bioactive compounds which are responsible for different pharmacological activities, that could be used in the treatment of infertility in the clinical trial.

Demerits: Long-term consumption of *C. lanatus* may cause gastrointestinal discomfort and allergic reactions while used in clinical application.

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Authors Contributions Statement

The experiment was designed, conducted, and written by Sandeep Kumar. Anima Tripathi: Conducted and interpreted the data. Poonam Singh: Oversaw experiments, analyzed data, and proofread the manuscript. After reviewing the results, all authors approved the manuscript.

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