RESEARCH PAPER

Fertility effects of phytochemical influences of Prosopis cineraria (Shami) in Swiss albino mice

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ABSTRACT

The current study sought to examine whether Prosopis cineraria (Shami) may help to enhance fertility level in Swiss albino mice. We picked 36 male mice (weight 30 ± 5 g) and put them into groups and subgroups. The control group was GrI, the Estradiol group was Gr2, the Estradiol and Prosopis cineraria (dose 150 mg/kg body weight) group was Gr3, and the Estradiol and Prosopis cineraria (dose 200 mg/kg body weight) group was Gr4. In the initial phase of this investigation, mice from groups Gr2, Gr3, and Gr4 received Estradiol of 25 µg/kg body weight dose for a period of 45 days. After the estrogen treatment was over, Gr2 was euthanized for study purposes while other mouse groups Gr3 and Gr4 were subdivided into three sub-groups (Gr3a, Gr3b, Gr3c and Gr4a, Gr4b, Gr4c respectively) administered P. cineraria extract as a treatment for 35 days to all plant parts (root, stem, and leaf) at two predetermined dosages. We examined sperm parameters like sperm count, morphology and motility along with hormone analysis like testosterone (T), FSH, and LH. The study indicates that Estradiol administered Gr2 showed significant changes in sperm count, motility, morphology and hormone parameters (testosterone, LH, FSH) compared to Gr1. Estradiol reduced sperm motility and FSH also. Significant effects were seen in all subgroups of Gr3 and Gr4 after the administration of P. cineraria extract. The subgroup Gr4 demonstrated significantly positive results compared to Gr3. The treatment with P. cineraria extract made a significant difference in all the measured parameters. When comparing two different doses of all parts of the same plant (root, stem, and leaf), 200 mg/kg body weight dosage was better for reproductive activity than the other dosage (150 mg/kg body weight). The study suggests that P. cineraria has properties that make it good for medicine that can help with fertility.

Keywords: Estradiol, Prosopis cineraria, LH, FSH, Testosterone, Mice.

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Introduction

Fertility achievement is the global health problem consisting about 15% of adults of entire issues, or about one in six. individuals encountering infertility during their lives (Luo, et al., 2021). Infertility is characterized by when couples are unable to conceive after one year of consistent intercourse. The most recent study from the World Health Organization (WHO) reported that around 50–80 million people around the world are infertile due to Male factors believe to be responsible for 50% of entire cases of infertility. Low sperm production, poor sperm motility, and aberrant sperm function are all common reasons why men cannot have children. Globally, decline in sperm parameter are the concerned issues in scientific world. In Western countries, sperm counts have also dropped by 1% over the past 50 years (Yang et al., 2010). There are several possible causes for these, including genetic problems, hormonal imbalances, and health problems including diabetes or infections. Lifestyle variables, such as smoking, alcohol consumption, and exposure to environmental contaminants, might adversely affect male fertility (Feinberg, & Kashanian, 2019).

In recent century many therapies on male infertility were investigated and several are in the market as different brand name. Several antioxidants, dietary methods, and medicinal plants have been suggested as possible treatments for male fertility difficulties (Roozbeh & Mortazavian 2017; Kumar et al., 2015; Singh et al., 2010). Various therapeutic herbs exhibiting anti-fertility or fertility-enhancing properties have been historically assessed to modulate male fertility globally. Modern scientists have also been interested in the fertility-related features of these plants.

As more men become interested in herbal remedies for infertility, supplementary approaches to treating infertility have also become more popular (Yao & Mills 2016). The European Association of Urology has recently said that using supplementary treatments based on traditional medicine is a multifaceted integrative strategy to treating male fertility issue. Health professional along with Internation medical institution encourage people to use medicinal plants and told academics to figure out how to use them in a way that makes sense to find new ways to cure infertility. Scientists are working to make clear how medicinal herbs affect male fertility because more people are interested in them. Some of these plants improve sperm count and motility, while others change how the testicles release hormones, according to the research that is out there. Some plants have flavonoids and phenolic chemicals that work as strong antioxidants against free radicals that have oxygen in them. They really do shield the sperm from free radicals and make the quality of the sperm and the chances of getting pregnant better (Rekka et al., 1996). Nevertheless, precise utilization of herbal medicines and the scientific understanding of their active molecular properties remain limited. So, it is important to look into how physiologically herbal phytocompounds solve the issues of male factors and to find natural compounds that have estrogenic and anti-estrogenic effects.

Prosopis cineraria (Shami) are from the "Fabaceae" family and it is a miraculous medicinal herb with many healing powers. According to Ayurveda and other traditional medicine systems, this plant is often used to treat a wide range of human illnesses (Lalitha and Kala; 2020). *P.cineraria* (Shami) contain many phytoconstituents, including tannins, steroids, flavone derivatives, and alkaloids (Girase and Talele; 2015). The extracts from the pods and leaves of *P.cineraria* (Shami) exhibit characteristics that fight cancer, diabetes, inflammation, and bacteria (Girase, et al., 2016; Chaudhary, et al., 2018). The stem and bark of this plant have anti-inflammatory, hypolipidemic, abortifacient, antioxidant, and antibacterial effects (Ahmed et al., 2019). Studies show that the bark, root, stem, and leaves of Shami contain a lot of nutrients that can aid with mineral insufficiency and enzymatic inhibitory actions (Mohammad et al., 2013). The chemicals listed above can make men more fertile by boosting antioxidant activity, stopping oxidative stress, lipid peroxidation, and imbalanced ROS activities (Yadav et al., 2018). These medicinal plants help the hypothalamic-pituitary-gonadal axis work better in diverse ways, such as by improving the quality of LH and testosterone (Bhamidipalli, et al., 2017; Parihar, et al., 2021). So, the goal of this study was to find out if *Prosopis cineraria* (Shami) may help male fertility improvement by using an animal model that was given Estradiol.

Material and methods

Chemicals used

The Estradiol tablets, marketed as Estraheal 2, contain Estradiol Valerate 2mg and were acquired from Healing Pharma India Pvt. Ltd., India.

Prosopis Cineraria (Shami)

Prosopis cineraria (Shami) known as Shami Plant were verified by Senior Scientist Dr. K. M. Prabhu Kumar, Systematics and Herbarium Division, National Botanical Research Institute (NBRI), Lucknow, India.

Ethanolic extraction of *Prosopis cineraria* (Shami) plant part preparation

The *Prosopis cineraria* (Shami) plant was washed with distilled water and cleaned well to get rid of dirt and dust particles once it was identified. The plant's roots, stems, and leaves were blocked and stored separately to dry.

After drying, the root, stem, and leaves were ground up into a powder. Soaking the grinded section of the plant part in ethanol at for 48 hours for the next step. To make the final ethanolic extract, a separate solution was run through a vacuum evaporation process with the use of a rota-vapor. The final dosages for the animal research with the *Prosopis cineraria* (Shami) plant were determined as follows: 150 mg/kg and 200 mg/kg of body weight for leaves, 150 mg/kg and 200 mg/kg of body weight for stem, 150 mg/kg and 200 mg/kg of body weight for root; after titration.

Animals and animal ethics

The Institutional Animal Ethics Committee (IAEC) approved the plan for all studies under proposal number IAEC No. 2021/1A-06/10/21.where 24 male healthy Swiss albino mice ranging in age from 8 to 12 weeks and weighing 30-35g, were procured from the Mahavir Cancer Sansthan and Research Centre, Patna, India (IAEC. No. 1129/PO/ReBi/S/07/CPCSEA), Animal house at a constant temperature of 22 ± 2 ºC, with a cycle of day and night, and were given food and water that was good for them.

Grouping animals

Gr1: Control

Gr2: Estradiol administered (25 µg/kg body mass)

Gr3: Estradiol pre-treated with Prosopis *Cineraria* (150 mg/kg b.w)

Gr4: Estradiol pre-treated with Prosopis *Cineraria* (200mg/kg b.w).

Gr1, Gr2, Gr3and Gr4 are the groups.

Experimental design

In accordance with the experiment, Gr2, Gr3, and Gr4 mice received Estradiol therapy with dosage of 25µg/kg b.w for 45 days. After the dose period was over, Gr2 was scarified for testing. For 35 days, the other groups, Gr3 and Gr4, kept getting *Prosopis Cineraria* in two different amounts. After giving *Prosopis cineraria* to the Gr3 and Gr4 mice, they were also scarified so that they could be examined.

Sample collection

The decapitation procedure was used to sacrifice the animal in each group. The collected blood sample from trunk was stored at 4^0C, and after that centrifuged for 15 minutes at 3000 g. For the LH, FSH, and testosterone, separate the serum in an eppendorf tube and freeze it at -20ºC.

Sperm count

During the dissection the cauda epididymis of all groups of mice was sliced and cleaned properly with 0.85 % normal saline. The clipped and cleansed cauda epididymis were made many punctures within a watch glass containing 1 cc of distilled water to facilitate the release of sperm. Furthermore, few drops of eosin Y were combined in gentle way. We carefully poured the samples into Neubauer's chamber and looked in 450X magnification.

Sperm motility

Every group of cauda epididymis was dissected precisely and prepared for the observation at 40X on phase contrast Microscope.

Sperm morphology

Sperm morphology was observed by the Haematoxylin and Eosin staining method, involving smear preparation, fixation, and staining. After staining, the slides were left to dry, and then sperm were analysed under a microscope.

Hormonal assay

All male hormones as LH (Luteinizing hormone), FSH (Follicle Stimulating Hormone), and Testosterone were measured by the ELISA method. ELISA kit commercially available by named "Immuno Tag," under brand of "Geno Technology" Inc. USA.

Histopathological Examination

The testes of every experimental group of mice were preserved in 10% formalin. After being dried with different concentration of ethanol, the tissues were embedded in paraffin with rotatory microtome (model HM 340E Microtom, Thermo Scientific, USA), the tissue sections were cut to a thickness of 5µm and subsequently Haematoxylin and Eosin (H&E) were used as a stain so that histopathological changes could be examined under a microscope.

Statistical analysis

ANOVA was used for the sperm motility, sperm morphology, sperm counts, and hormonal levels. Tukey's test performed multiple comparisons used to find results that were statistically significant P -value ($p<0.05$) using GraphPad Prism5 software (GraphPad Software, Inc., San Diego, USA) was used to statical analysis.

Results

This study examined phytochemical effects of *Prosopis cineraria* at two distinct dosages, utilizing all plant parts (root, stem, and leaf) after Estradiol treatment in a mouse cohort, resulting in significant alterations in many parameters, as delineated in Table 1.

Dose: (Gr3) 150mg/kg body weight (a- Root, b- Stem, C- Leaf)

	Sperm Parameters					
Mice Group	**oun t 0⁶/ nl)**	**otil ty %)**	**orp log (%)**	**stos rone g./m**	**SH g./ nl)**	**H g. nl)**
r1	21 03	.68).02	.05).09	88 ± 01	79 01	12 00
r2	193).04	.51).03	.62 0.3	73 ± 05	61± 006	04 00
r3	817).02	.72).09	.86).07	93 ± 01	10 04	24 00
r3	628).02	.37).03	.39).15	51 ± 03	97± 005	12 02
r3	298).03	.14).02	.06).09	84 ± 01	53).07	86 05

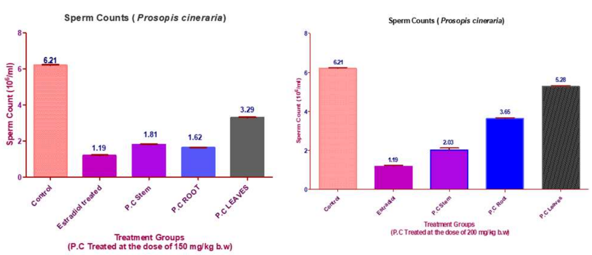
Table-1: Corelation of Sperm Count, Motility, Morphology, and hormones at 150mg/kg body weight

Dose: (Gr4) 200mg/kg body weight (a- Root, b- Stem, C- Leaf)

	Sperm Parameters					
Mice Group	**oun t 10⁶/ nl)**	**ot ty %)**	**orp logy %)**	**stos rone g./m**	**S H g. nl)**	**H g. nl)**
r1	21 ± 03	.6 ± 02	.05± 09	88 ± 01	79 01	12 01
r2	193).04	.5 03	.62 0.03	73 ± 05	61 06	04 05
r4	)35).09	.2 ± 07	).11 0.07	52 ± 08	90 03	29).0
r4	635).03	.5 ± 17	.92 0.07	65 ± 02	96 05	43 05
r4 c	283 0.04	.4 ± 07	).67 0.07	27 ± 03	59 05	12 02

Table-2: Corelation of Sperm Count, Motility, Sperm Morphology, and hormones at 200mg/kg body weight

Effect on Sperm Counts



In this study it was observed that estradiol treated mice had sharply reduced the sperm counts compared to the control. However, *Prosopis cineraria* treated group showed significant (p<0.001) increase in sperm counts. Gr4c (leaves) of Gr4(200 mg/kg body weight of *P. cineraria*) marked significantly increase and effective than Gr3(150 mg/kg body weight of *P. cineraria*) (Figure -3).

Effect on sperm motility

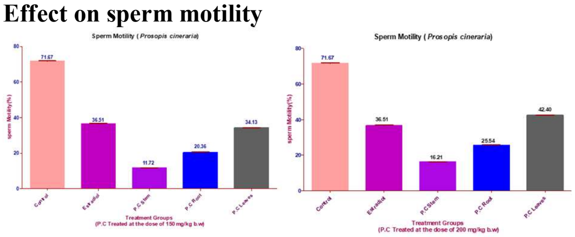


Figure-4: Corelation of sperm motility in different group of mice (n=6; mean ± SD, significance at p<0.001). (Gr4c - leaves at dose 200mg/kg body weight) (Gr3 – leaves, dose 150mg/kg bw).

Estradiol treated mice showed reduction in the sperm motility compared to the control. However, *Prosopis cineraria* treated group showed significant (p<0.001) increase in sperm motility. Gr4c (leaves) of Gr4(200 mg/kg body weight of *P. cineraria*) marked significantly increased sperm motility than Gr3(150 mg/kg body weight of *P. cineraria*) (Figure -4).

Effect on sperm morphology

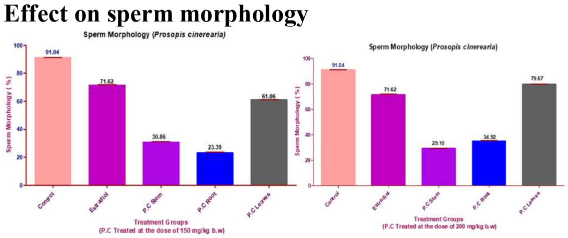


Figure-5: Corelation of sperm morphology in different group of mice (n=6; mean ± SD, significance at p<0.001). (Gr4c – leaves, dose 200mg/kg bw) (Gr3 – leaves, dose 150mg/kg bw).

The elevated dose 200 mg/kg body weight of *P. cineraria* (Shami) demonstrated an increase in the sperm morphology (Figure 5; p<0.001). In comparison to Gr3, Gr4 (with a dose of *P. cineraria* at 200 mg/kg body weight), particularly Gr4c(leaves) showed greater efficacy. The estradiol-treated group showed lower sperm morphology (p<0.001) as compared to the control (Figure -5).

Hormonal analysis

After administered of *P. cineraria*, the level of testosterone was dramatically elevated (p<0.001), indicating a stabilization of gonad function (Figure -6) while the estradiol-treated mice exhibited a substantial decrease (p<0.001). In comparison to the control group in comparison to Grr3 (administered of *P. cineraria* at 150 mg/kg body weight) and G4 (administered of *P. cineraria* at 200 mg/kg body weight), Gr3c (leaves) showed superior efficacy over Gr4 (Figure -6).

Testosterone (T)

Testosterone (T) Compared to control group the testosterone (T) tested estradiol-treated mice group had showed significant (p<0.001) reduction. After administration of *P.Cineraria* the level of testosterone was significantly (p<0.001) increased, denoting normalization in the endocrine function (Figure -6). Compared to Gr3 (administration of *P.Cineraria*, at 150 mg/kg body weight) vs. Gr4 (administration of *P.Cineraria*, at 200 mg/kg body weight); G3c (leaves)G3 was highly effective than Gr4.

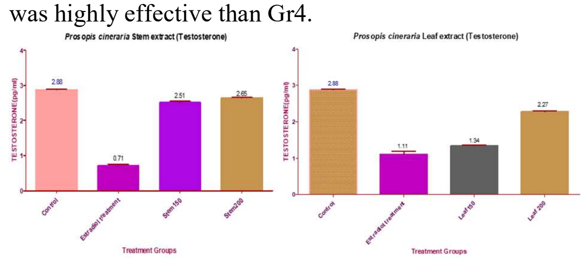


Figure -6 Corelation of Testosterone level in various groups of *P. Cineraria.*, (n=6; mean ± SD, significance at p<0.001). (Gr4c – leaves, dose 200mg/kg b.w) (Gr3 – leaves, dose 150mg/kg b.w).

Luteinizing Hormone (LH)

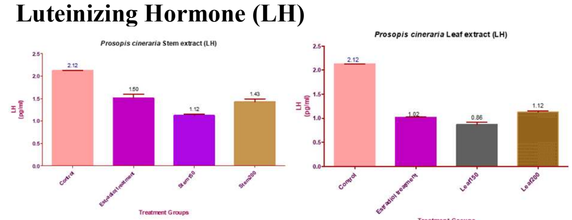


Figure -7 Corelation of LH in various groups of *P. Cineraria* expressed (n=6; mean ± SD, significance at p<0.001). (Gr4c – leaves, dose 200mg/kg bw) (Gr3 – leaves, dose 150mg/kg bw).

The elevated dose 200 mg/kg body weight of *P. cineraria* (Shami) demonstrated an increased level of Luteinizing Hormone (Figure 7; p<0.001). In comparison to Gr3, Gr4 (with a dose of *P. cineraria* at 200 mg/kg body weight), particularly Gr4c(leaves) shown greater efficacy. The estradiol-treated group showed lower Luteinizing Hormone (LH) (p<0.001) as compared to the control (Figure -7).

Follicle Stimulating Hormone (FSH)

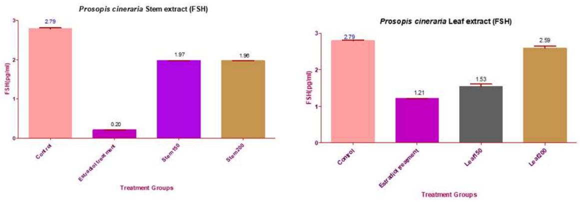


Figure -8 Corelation of FSH in various groups of *P. cineraria* expressed (n=6; mean ± SD, significance at p<0.001). (Gr4c – leaves, dose 200mg/kg b.w) (Gr3 – leaves, dose 150mg/kg b.w).

The elevated dose 200 mg/kg body weight of *P. cineraria* (Shami) demonstrated an increase the level of Follicle Stimulating Hormone (FSH) (p<0.001). In comparison to Gr3, Gr4 (with a dose of *P. cineraria* at 200 mg/kg body weight), particularly Gr4c (leaves) shown greater efficacy. The estradiol-treated group shown lower Follicle Stimulating Hormone (FSH) as compared to the control (Figure -8).

Histopathological Analysis

In the control testis of mice, the main primary Spermatocytes, Spermatogonia, Spermatids (SPM), and Spermatozoa are well organised in the Seminiferous Tubules (ST).

In spermatogenesis, the Leydig cells that line up the seminiferous tubules are working perfectly, as seen in (Figure -9A). After estrogen therapy, the testicular sections exhibit impaired functions, characterized by the presence of just the least active spermatogenetic phases, reflecting merely 30 % of total (Figure -9B). Furthermore, there is haemorrhage in the Leydig cells, indicating that they are also in a highly deteriorating condition. However, after administered *P. cineraria* at a dose of 150 mg/Kg b.w., A significant improvement has happened because the spermatogenetic phases are back to their normal condition - spermatozoa, spermatids, spermatogonia, and primary spermatocytes indicate that cellular activity has returned to normal (Figure -9C). Additionally, the normal function of Leydig cells is restored. Nonetheless, *P. cineraria* at a dosage of 200 mg/kg b.w, show small improvement on Spermatogenesis, particularly in the initial spermatocytes, whereas the spermatogonia, spermatids, and spermatozoa exhibit significant restoration (Figure -9D).

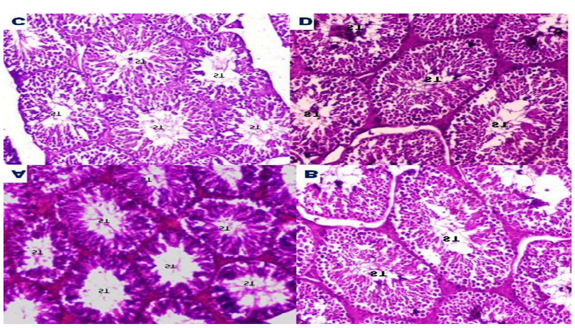


Figure 9: In this microphotograph of [A], the sections of the control testis stained with Haematoxylin and eosin, figure shows the normal arrangement of the spermatogenetic stages (ST) at 500x magnification. [B]. Estradiol-treated mice testis section exhibits markedly diminished spermatogenetic stages (ST) and abnormal arrangement of seminiferous tubules at 500x magnification. [C] The testis of mice administered *P. cineraria* at a dosage of 150mg/Kg b.w restored function during spermatogenesis stages 500x. [D] The testis of mice administered with *P. cineraria* at a dosage of 200mg/Kg b.w exhibited minor restoration 500x.

Discussion

Estradiol is the most active form of estrogen that works in males. ERβ and ERβ are enzyme which conversion is from testosterone to estrogen (Bendis et al., 2024; Fietz et al., 2014; Oliveira et al., 2014; Bernardino et al., 2016). It acts as both inhibitory and stimulating effects. Stops Leydig cells from growing again and stops these cells from making testosterone (Hess et al., 1997). Estradiol is acknowledged for its capacity to regulate multiple aspects of spermatogenesis in testicular cells, including germ cell proliferation. Proliferation, differentiation, survival, and apoptosis (Fujikura & Fujinoki 2024; ; MacCalman et al., 2017; Chimento et al., 2010 & 2014; Pentikainen et al., 2000). It synthesized in different types of epithelial cells, such as spermatozoa, immature germ cells, Leydig cells, Sertoli cells, and the proximal duct of the epididymis. In current study Spermatogenesis in testicular seminiferous tubules was markedly compromised in the current study, as a result it led to azoospermia.

The hypothalamic-pituitary-testosterone axis regulates the secretion of growth hormone (GH), luteinizing hormone (LH), and testosterone. Attached to receptors in the anterior pituitary releases FSH and LH into both gonadal and non-gonadal tissues. The Leydig cells in the testes make androgen s that stop the pituitary gland and the central nervous system from making FSH and LH, respectively.

Androgen's negative feedback effect makes the pituitary less sensitive to gonadotropin-releasing hormone, which lowers the level of LH in the blood and the size of the LH pulse (Widyastuti et al., 2020). A rise in testosterone is essential for sperm quality and quantity. Testosterone regulates spermatogenesis through the phosphorylation of the cAMP response element-binding protein. Our finding revealed that, unlike control group, the treated animals exhibited a significant reduction in testosterone levels, followed by a trend indicating decreased FSH and LH levels. Figure 6 indicates that a decrease in testosterone may have influenced Leydig cell activity; however, this alteration did not lead to the reduction in FSH and LH through a negative feedback mechanism (Chandrakant et al., 2018).

Current finding shows that the testosterone deficiency was mostly caused by Estradiol acting directly on the testicles. But all the measured the parameters underwent significant enhancement following treatment with *P. cineraria* extract. When comparing two doses of the same plants of all parts—root, stem, and leaf—the 150 mg/kg body weight dose had a better effect on reproductive ability than the 200 mg/kg body weight dose. The data indicate that *P.cineraria* may have medicinal use for enhancing fertility.

P. cineraria (Shami) contain many phytoconstituents, including tannins, steroids, alkaloids, and flavonoid derivatives (Girase and Talele 2015), which are essential for boosting fertility in the Estradiol-induced model. The *Prosopis cineraria* extract contains several flavonoid and steroids (Garg and Mittal, 2013). Kang et al. (2010) suggest that flavonoids may have enhanced the effects of steroids by augmenting male sex hormones (Ramalingam et al., 2020). Taking everything into account, it's possible that the flavonoids in the extract improved sperm parameters by acting as antioxidants.

Conclusion

By the entire study *P. cineraria* extract made all the studied metrics much better. The dose of 200 mg/kg body weight had the best effect on reproductive processes when compared to the dose of 150 mg/kg body weight. This was true for different parts of the same plant (root, stem, and leaf). So, *Prosopis cineraria* could be suggested as a treatment for fertility problem.

Acknowledgement

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Author Contributions

The experimental work was conceived by P.K, A.K and P.M. Sampling for the fieldwork (plant collection) was conducted by P.K. The majority of the writing activities for the manuscript were done by P.K with additional support from A.K and P.M. Animal experimentation was done by P.K. Literature search was conducted by P.K and K.S. The design and creation of the figures was carried out by P.K. Data curation and statistical analysis was done by P.K and K.S. The final manuscript was reviewed and endorsed by all authors.

Data Availability

The datasets evaluated in the present research are not publicly accessible owing to a lack of participant agreement to disclose data outside of the team of investigators, but they are available from the corresponding author upon reasonable request.

Declarations

Consent to participate

For any of the clarification related to the publication of the article, the authors provide the consent.

Consent for publication

Not applicable.

Conflict of interests

The authors declare no competing interests.

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