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Assessment of the antifertility potential of Silver nanoparticles synthesized using *Pongamia pinnata* leaf extract in male wistar rats for the development of a male contraceptive agent

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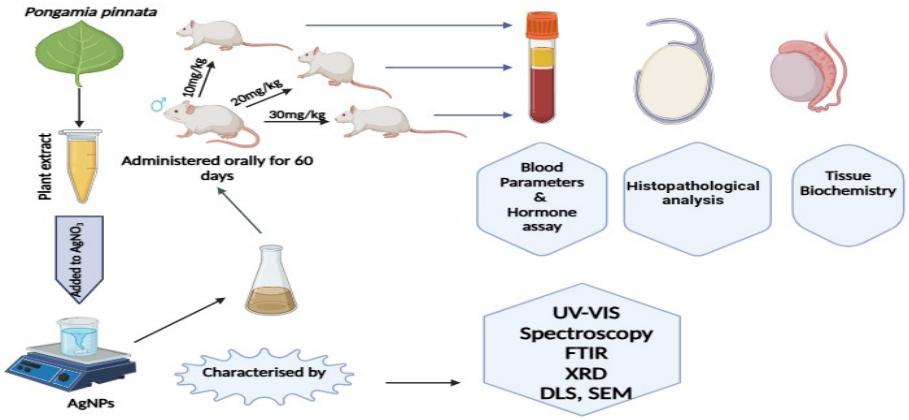
Article

ABSTRACT



Plant-mediated silver nanoparticles (AgNPs) possess a variety of biological properties, such as antibacterial, anti-inflammatory, and antifertility properties. The plant we used in this study is *Pongamia pinnata*, which have multiple medicinal values. Nevertheless, the possible antifertility functionality, although having discovered few works which combine it in synthesizing AgNPs, has been proven to be limited. In the present investigation, for the production of AgNPs, methanolic leaf extract was used from *Pongamia pinnata*. Male Wistar rats were divided into 5 groups, a control group, 3 experimental groups that received different concentrations of the AgNPs, and a recovery group. For 60 days, doses were administered orally. The concentration, progression of the sperm, serum testosterone concentration and histopathological examinations of testicular tissues were assessed. Sperm analysis of the rats in the AgNP-treated groups reduced their sperm motility more than control group and had significantly lower serum testosterone levels at different doses tested, particularly in the high-dose cohort. Under the light microscope, histopathology changes indicated that the germinal epithelium was disrupted, and there were shrunken seminiferous tubules and decreased spermatogenesis. These observed antifertility effects in AgNP groups might mean that AgNPs, along with bioactive compounds of *Pongamia pinnata*, affect spermatogenesis and male reproductive hormones. The results further suggest a positive relationship between dosages and the manifestation of such reproductive changes.

Keywords- Pongamia pinnata, silver nanoparticles, antifertility, histopathological, testosterone

INTRODUCTION

India's rapid population growth is one of the major issues facing developing nations like it. Aside from negatively affecting our economic strategies, this population boom will throw off the equilibrium of our socioeconomic infrastructure.[1] Extensive research highlights the unfulfilled need for safe, affordable, and widely accepted contraceptives to avert unintended pregnancies and consequent abortions. Since the beginning of recorded history, there has been an effort for an oral contraceptive that can regulate human reproduction.[2] In the past few decades, the scientific area of reproductive medicine has been developed by dealing with the male and female reproductive system and relevant clinical problems, for example, infertility, puberty, menopause, and STIs.[3] Present-day contraceptives, therefore, have input in reproductive planning; however, a large population must still depend on traditional medicine for fertility regulation, demonstrating how these natural products remain up to date.[4] It is critically important to locate some secure and reliable herbal contraceptives. Even ancient societies' primary members used herbal contraceptives for regulating fertility as well as preventing getting conceived.[5] Antifertility medicines, generally associated with oral contraceptives, are treatments that suppress fertility.[6] In recent years, nanotechnology has presented new nanoparticle uses in disease probing, cures, and gene transport.[7]

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In contrast to traditional techniques of nanoparticle synthesis, which require high temperatures and pressures as well as toxic reagents, the newly developed approaches called “green synthesis” involve the utilization of plants. This nanoparticle preparation method is short and free of hazardous residues.[8] Particles as small as nanoparticles may traverse biological membranes and access even the smallest capillaries in the body (such as the blood-brain and blood-testes). Size, shape, and surface area are acknowledged to be crucial factors that contribute to a nanoparticle's toxicity.[9] It is growing in popularity that reproductive toxicity plays a key part in general toxicology. The necessity of raising public knowledge of NPs' toxicity to the reproductive system is underscored by the fact that fertility, reproduction, and foetal development are vital to a species' survival.[10] Integrating plants and microbes for the production of nanoparticles is commonly referred to as "green synthesis".[11] Of the metal nanoparticles, the silver nanoparticles (AgNPs) are the most attractive due to their exciting physical and chemical properties that make them useful in clinical applications.[12] AgNPs may potentially impede reproductive processes because they have been observed to adversely affect mammalian germ cells.[13] The current work aims to prepare AgNPs following the biological reduction by *Pongamia pinnata* leaf extract and assess the potency of synthesized AgNPs on male Wistar rats' fertility. Several methods were used to characterize the AgNPs, such as UV-VIS spectroscopy, FTIR, DLS, ZETA potential, XRD, EDX, and SEM.

Pongamia pinnata (L.) Pierre, k/a *Pongamia glabra* or ‘Karanj’ has long been used in Ayurveda for its antihelminthic, alexipharmic, and other therapeutic uses for skin, eye, and genital tract infections.[14] It is also equally famous for its use in managing diabetes mellitus. Specifically, in many developing countries where the accessibility to formal healthcare services might be considerably restricted, classical phytomedicines remain the only therapeutic remedy for several diseases.[15] *Pongamia pinnata* has been employed in India and neighboring countries as a fish poison, timber, fuel, animal feed, and medicinal plant. It is well documented that many of the pharmacological activities of medicinal plants are due to their antioxidant phytochemicals, including polyphenols, flavonoids, phenolic acids, and carotenoids. These bioactive molecules are vital for decreasing oxidative stress and avoiding chronic diseases.[16] Many other types of chemicals, including terpenoids and flavonoids, emerged as a result of the phytochemical investigations. This plant shows an extensive spectrum of biological activity, pursuant to the pharmacological research.[17] Some researchers have established that *Pongamia pinnata*'s methanolic extract has antibacterial properties against many microorganisms like *Staphylococcus aureus*, *E.coli*, *Candida albicans*, and so on.[18]

MATERIALS AND METHODS

Plant Collection and Authentication

The *Pongamia pinnata* (*P. pinnata*) plant was collected from around the University of Rajasthan and authenticated at the Department of Botany, University of Rajasthan, Jaipur. The herbarium sample was assigned the identification number RUBL21227.

Animal Model

Good adult male albino Wistar rats weighing 150–200 grams, aged 4-5 months, were purchased through Lala Lajpat Rai University of Animal and Veterinary Sciences (LUVAS), located in Hisar, Haryana. The animals were kept in polypropylene cages of 430 x 270 x 150 mm in controlled environments with a 12-hour day light and darkness cycle. Rats were fed a diet of regular rat chow with soaked wheat and gram added, and they were given unlimited access to water.

Treatment Protocol

The experimental protocol involved five groups, each containing eight rats:

Group I (Control Group): Distilled water was used for treatment.

Group II: Treated with AgNPs at 10 mg/kg body weight.

Group III: Subjected to 20 mg of AgNPs per kilogram of body weight.

Group IV: Treated with AgNPs at 30 mg/kg body weight.

Group V (Recovery Group): AgNPs (20 mg/kg body weight) were administered for 60 days, after which there was a 30-day recuperation period during which no medication was administered.

The II, III, and IV groups received AgNPs for 60 days, while Group V was assessed for reversibility after an additional recovery period. The recovery period of 30 days was chosen for several reasons which include tissue repair, hormonal balance, inflammation resolution, and drug elimination as 30 days allows the elimination of most drugs and their metabolites from the body of animals. The middle dose was chosen for recovery because it is scientifically optimal and ethically sound, enabling clear observation of recovery while avoiding the extremes of negligible or severe toxicity.

Fertility Test and Sacrification Schedule

After the dosage period, male rats that had received treatment were paired with females at a 1:2 ratio for post-mating fertility assessment over five days. Following this, animals were sacrificed under light ether anesthesia.

Study Parameters

The study evaluated several parameters, including body and organ weight, sperm motility and density, tissue biochemistry, hormonal assays, and histopathological changes. For sperm motility and density one milliliter of physiological saline was combined with fifty milligrams of cauda epididymis tissue to test sperm motility and density. Five minutes later, one area of a glass slide had a single drop of the mixed sample on it, and similarly, another section had a cover slip. The percentage of motile sperm was determined according to Kumar et al. (1989) by calculating the number of immotile and moving sperm heads per unit area.[19] The organs of reproduction, the ventral prostate, seminal vesicles, testes, and epididymides, were removed with precision, washed in saline solution, placed in vials, and stored at - 20ºC for biochemical examination. Several biochemical meters were used to evaluate the effect of AgNPs on tissue health activity. According to Lowry et al. (1951),[20] the protein concentration was

estimated, and cholesterol content was calculated based on Mann (1964).[21] The glycogen was estimated using Montgomery's (1957)[22] procedure, while the quantities of sialic acid and fructose were estimated using Foreman et al., 1973. Furthermore, adrenocortical ascorbic acid was determined using the Foreman protocol technique to measure any eventual system impact.[23] Serum testosterone level was analyzed by using an ELISA kit (WHO, 1999).[24] For histopathological analysis, the contralateral side of the testes was preserved in Bouin's solution, with all adhering tissue carefully removed. The tissue samples were then dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 6 µm thickness were cut and subsequently stained with Harris's hematoxylin and eosin to observe any histological changes.[25]

Statistical Analysis

The Student's t-test was used to examine the data, which were presented as mean ± standard error (SE). A p-value of less than 0.05 was considered statistically significant, while a p-value of less than 0.01 was considered highly significant.

RESULTS

The results demonstrated that the administration of silver nanoparticles (AgNPs) synthesized from *Pongamia pinnata* leaf extract significantly affected reproductive parameters in Wistar male rats. Interestingly, animals given AgNPs displayed a decrease that is dose-dependent in both the body and reproductive organ weights, with the highest dose group exhibiting the most pronounced effects.

Weight of the Body and Organs

Reproductive organs, such as weighing were done on the testes, seminal vesicle, ventral prostate, epididymides, and vas deferens. following the recording of the initial and final body weights. removing excess fat. Weights were measured using a milligram balance, providing quantitative data on the effect of AgNPs on organ weight reduction (Table 1).

Table 1 Changes in Body and organs weight after treatment with AgNPs of *P. pinnata* for 60 days

Treatment groups	Group-I	Group-II	Group-III	Group-IV	Group-V
Initial B.wt	142.50± 4.53	146.25± 5.32^{ns}	143.75± 3.23^{ns}	145.00± 3.77^{ns}	145.00± 3.27^{ns}
Final B.wt	182.50± 3.65	186.25± 3.23^{ns}	188.75± 4.40^{ns}	185.00± 3.77^{ns}	188.75± 3.50^{ns}
Testes	1383.59± 3.61	1358.08± 3.55**	1355.31± 2.99**	1353.30± 4.31**	1378.76± 3.22^{ns}
Epididymides	565.74± 5.03	542.48± 4.84*	540.04± 4.57**	531.29± 4.20**	556.00± 5.84^{ns}
Seminal Vesicle	402.91± 2.73	377.42 ± 1.28**	377.78 ± 4.60**	369.06 ± 4.31**	388.55 ± 4.27^{ns}
Ventral Prostate	110.01± 3.02	93.70± 1.42*	91.03± 2.91**	87.35± 3.59**	105.98± 5.07^{ns}
Vas Deferens	188.80± 3.66	166.19± 4.77*	162.77± 5.33**	153.75± 6.44**	169.40± 4.74^{ns}

(Mean ± SEM of 8 animals) Treated Group II, III, IV, and V compared with Control Group I. *= Significant (p≤0.05), **= Significant (p ≤0 .01), ^{ns}= non-significant

Sperm Density and Motility

After performing sperm motility and density parameters following results were seen. (Figures 1 & 2).

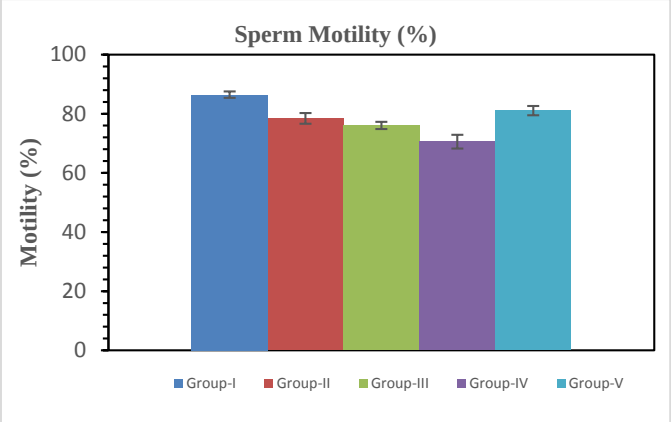

Figure 1. Changes in sperm motility after treatment with AgNPs of *P. pinnata*

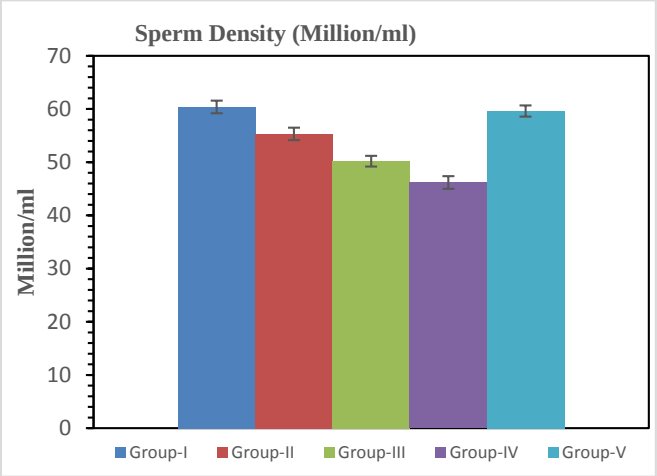

Figure 2. Changes in sperm density after treatment with AgNPs of *P. pinnata*

Tissue Biochemistry

Biochemical parameters were performed for fructose (seminal vesicle), cholesterol (testis), glycogen (testis), sialic acid (epididymis), ascorbic acid (adrenal gland), and total protein (testis). The biochemical analyses for the subjects are summarized in Table 2 below.

Treatment groups	Fructose (mg/gm)	Cholesterol (mg/gm)	Glycogen (mg/gm)	Sialic acid (mg/gm)	Ascorbic acid (mg/gm)	Total protein (mg/gm)
Group-I	6.35 ± 0.11	9.44 ± 0.20	6.66 ± 0.25	8.50 ± 0.18	3.37 ± 0.15	268.23 ± 9.12
Group-II	5.3 ± 0.16**	7.08 ± 0.20**	5.33 ± 0.50*	7.25 ± 0.41*	2.31 ± 0.09**	242.44 ± 3.76*
Group-III	4.05 ± 0.14**	5.97 ± 0.20**	3.08 ± 0.12**	5.37 ± 0.18**	1.75 ± 0.09**	226.48 ± 5.96**
Group-IV	3.10 ± 0.10**	4.72 ± 0.27**	1.75 ± 0.12**	4.37 ± 0.18**	1.18 ± 0.09**	201.36 ± 2.87**
Group-V	5.85 ± 0.07^{ns}	8.47 ± 0.29^{ns}	5.83 ± 0.37^{ns}	8.00 ± 0.26^{ns}	3.00 ± 0.16^{ns}	267.06 ± 6.19^{ns}

(Mean ± SEM of 8 animals) Treated Group II, III, IV, and V compared with Control Group I. *= Significant (p≤0.05), **= Significant (p≤0 .01), ^{ns}= non-significant.

Hormone assay

Serum testosterone was measured by using an ELISA kit. A significant reduction in hormone levels in comparison to control was observed. (Figure 3)

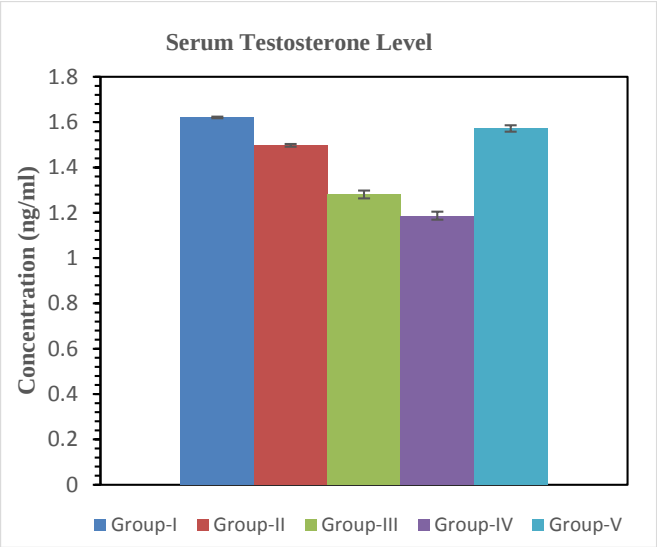

Figure 3. Level of serum testosterone after treatment with AgNPs of *P. pinnata*

Histopathological Study

In the control group (Figure 4), the histoarchitecture of the testes displayed an intact germinal epithelium and normal seminiferous tubules, encompassing all stages of spermatogenesis. However, in AgNP-treated groups (II, III, IV), there was evident damage to the germinal epithelium along with irregularities in the lumen of the seminiferous tubules. Interestingly, the recovery group (V) showed normal interstitial cells and seminiferous tubules, similar to the control group, indicating a degree of tissue restoration post-treatment.

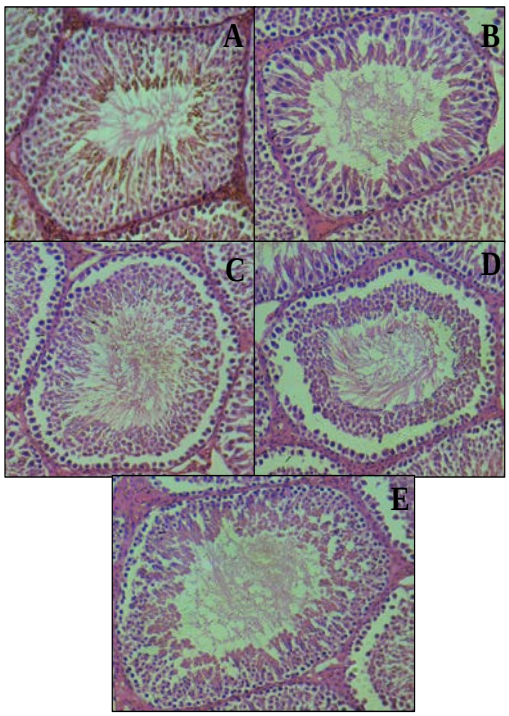

Figure 4. (A) Photomicrograph of Control group (B) Photomicrograph of Group-II (C) Photomicrograph of Group-III (D) Photomicrograph of Group-IV (E) Photomicrograph of Group-V

DISCUSSION

The current research focuses on acute and chronic dosages of AgNPs derived from *Pongamia pinnata* on male rat reproductive performance. It was evident from the male rats' declining reproductive organ weight that the extract altered the structure and function of the testes, epididymis, seminal vesicle, ventral prostate, and vas deferens.[26] Specific androgen receptors, which are mostly reliant on testicular androgens, can be observed in the prostate glands of rats.[27] As the results show, there is a decline in cholesterol level, a precursor molecule for the synthesis of testosterone, which is also reducing significantly. This implies that the weight of the testes might be due to an insufficient supply of androgens.[28] Male infertility is associated with chronic inflammatory disorders that show up in the body, like obesity, metabolic syndrome, and oxidative stress caused by excessive ROS creation. One therapy option for males with subfertility is to prevent ROS generation.[29] This study focuses on different doses of AgNPs in male rats through the oral route. The present study intimates that AgNPs have detrimental effects on sperm quality, characterized by motility and concentration.[30] The study also declared a decrease in sialic acid content after using AgNPs, which may in some way impact the motility and fertilization ability of sperms. The acrosomal membrane has to possess proper sialic acid since it is vital in multiple steps of the fertilization process.[31,32] The fact that treated rats' levels of adrenal ascorbic acid decreased may be of particular interest. As a potential boost to fertility, ascorbic acid is a vital biochemical element in the reproductive process.[33] Testicular glycogen might have decreased as an outcome of fewer spermatids, the post-meiotic germ cells where glucose metabolism takes place.[33] Testicular protein concentration drastically dropped after AgNPs of P.pinnata leaf extract were administered, most likely as a result of the testes' lack of spermatogenic phases.[28] One possible explanation for the decrease in fructose concentration in seminal vesicles is a decrease in gland secretory activity, which in turn indicates a reduction in the supply of androgen.[33]

Consistent with previous studies, the present research emphasized the toxic effects of AgNPs on the reduction of Sertoli and Leydig cells, which may contribute to decreased testosterone levels, as indicated by the testicular histology of the rats.[34] Decreased Leydig cell count may reduce testosterone levels, which help mature spermatozoa.[35,36] High testosterone levels in the testes may also reduce secondary spermatocyte and spermatid counts, essential in sustaining the functionality of the male organs of reproduction.[25,37] These highlight the disruption of androgen-dependent functions because testosterone is crucial in spermatogenesis, libido, and structural integrity of the reproductive organs. The motility of sperm in the cauda epididymis, crucial for fertilization, could be affected by a

reduced testicular content, as shown in the study.[31] The anti-spermatogenic nature of the extract was established by histopathological studies in rats treated with it, which displayed abnormalities in seminiferous tubules and a decrease in spermatogenic factors and spermatozoa in the testis.[38] Rat testicular cellular architecture had degenerative changes when Ag-NP was given to them in contrast to the control. These earlier degenerations are lines of evidence that indicate the nanoparticle could trigger oxidative and cellular damage to the testes of rats.[13]

CONCLUSION

This study demonstrates that silver nanoparticles (AgNPs) synthesized from the methanolic leaf extract of *Pongamia pinnata* exhibit antifertility effects in male Wistar rats. Using biogenic AgNPs offers the possibility to regulate fertility through the extract of the *Pongamia pinnata* plant using nanotechnology. The data indicate that these nanoparticles significantly influence sperm parameters, biochemical markers, testicular histomorphology, and hormonal profiles, suggesting a promising avenue for the development of non-hormonal male contraceptives. This finding validates the folkloric application of *Pongamia pinnata* in reproductive health and intensifies the prospect of creating non-hormonal male contraceptives with bio-friendly compounds. However, these results should be viewed as preliminary and hypothesis-generating. Further research is essential to elucidate the long-term toxicity, optimal dosing regimens, duration of effect, and the reversibility of the antifertility outcomes. This work also feeds into emerging science in nanomedicine for reproductive health to propel further development of new technologies in male contraception.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

DECLARATION

The appropriate Animal Ethics Approval or Institutional Animal Ethics Committee (IAEC) Approval ID: UDZ/IAEC/2023-1/02.

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