

# Green Synthesis of Silver Nanoparticles Using *Pongamia pinnata* Methanolic Extract and Their Antifertility Impact on Male Rats: A TEM Study

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## Research Article

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# Abstract

The present study explores the green synthesis of silver nanoparticles (AgNPs) using methanolic leaf extract of *Pongamia pinnata* and investigates their potential antifertility effects in male Wistar rats. The synthesized AgNPs were characterized by UV–Visible spectroscopy, FTIR, XRD, and SEM, confirming their nanoscale size, crystalline nature, and the presence of biofunctional groups responsible for stabilization and reduction. Male rats were orally administered AgNPs for 60 days, and testicular tissue was subsequently examined for ultrastructural changes using transmission electron microscopy (TEM) and histological analysis of epididymis y using light microscope.

TEM analysis revealed significant alterations in the seminiferous tubules, including disorganized germinal epithelium, vacuolization, and mitochondrial damage, indicating impaired spermatogenesis. Additionally, biochemical analysis of the reproductive tissues showed a marked decrease in total protein content compared to control groups, suggesting disruption of normal cellular metabolism and structural protein synthesis. These findings highlight the possible antifertility effects of biosynthesized AgNPs, mediated through oxidative stress and protein degradation pathways.

This study underscores the dual role of *Pongamia pinnata*-mediated AgNPs in nanomedicine and reproductive toxicology, providing insights into their biological impact and potential use in male contraception research.

## Introduction

Over the past few decades, nanomaterials (1–100 nm materials) have garnered a lot of interest in a variety of sectors, including biomedicine, energy storage, catalysis, and sensing, because of their distinct physicochemical characteristics in comparison to their bulk forms [1]. Focused on several facets of nanotechnology, the field of nanoscience differs from the wider world we are used to in our everyday lives. Metals are compact, which increases the number of atoms suitable for an object's surface. Amazing variation may be seen in nanoparticles' chemical makeup, size, charge, shape, and surface area (NPs) [2]. Silver nanowires and nanoparticles are taken into consideration among metals such as Pd, Cu, Au, Zn, Sn, Ag, and Co because of their notable electrical conductivity and localized surface plasmon resonance characteristics [3]. AgNPs have grown to be a sophisticated material of consumer interest, and their many uses in biomedicine and other fields are noteworthy. AgNPs have become more well-liked in a variety of industrial sectors, such as the food and textile industries, female and feminine hygiene products, cosmetics, water purification plants, healthcare products, and environmental pollution control. Moreover, AgNPs are widely employed in magnetic fields, thermal, electronics, engineering, and energy applications [4–5]. It is claimed that silver nanoparticles have antifungal, anti-inflammatory, antiviral, and antiplatelet properties [6]. However, AgNPs produced by biological and electrochemical methods have superior size stability, high solubility, and high productivity [7]. Since the green synthesis of nanoparticles avoids the hazardous materials often employed in chemical synthesis and instead makes use of non-toxic phytochemicals, it has become increasingly popular. Using extracts from various plant

components, microbial cells, and biopolymers, green synthesis techniques are categorized as such. As intended, the produced nanoparticles are biocompatible and possess the appropriate level of effectiveness [8].

The environmental impact is lessened by these eco-friendly methods, which also improve the nanoparticles' biocompatibility [9]. The plant *Pongamia pinnata* belongs to the Fabaceae family, which is widespread in India. In medical systems, several portions of this plant are employed as antiseptics, blood purifiers, and wound healers to treat a variety of illnesses [10]. The presence of flavonoids (flavones, isoflavones, and chalcones), protein, lipids, fiber, and carbs in the seed extracts provided medicinal potential in the treatment of infections and skin conditions, rheumatoid arthritis, hypertension, and lung cancer. The seed's high flavonoid content may make it useful as a metal-reducing agent in the formation of AgNPs [11]. Both rich and developing nations are extremely concerned about overpopulation these days. Among emerging nations, India is home to a large population, which is predicted to grow to 9.2 billion people by 2050 [12]. Half of all conceptions are thought to be unintended, and half of the subsequent pregnancies are thought to be unwanted. Failure to utilize contraception accounts for half of unwanted births in most circumstances, with the other half being caused by issues with using contraceptives or technique failure [13]. The use of contraceptives, an efficient method of birth control, has increased recently. Contraceptives for men are currently less effective than those for women. The majority of these contraceptives are synthetic and have serious adverse effects [14]. The majority of the cost of contraception is currently borne by women, with males having no practical or efficient alternatives except condoms and vasectomy. Because of the drawbacks of all birth control options, scientists are now trying to create an oral herbal contraceptive that is more reversible, effective, and acceptable to men [15]. The widespread use of plants and their products to regulate both male and female fertility may be attributed to their cost, accessibility, absence of negative medication reactions, and—most importantly—protection of user privacy. Therefore, the development of a plant-based oral active male contraceptive method has a promising future [16]. Among the alkaloids found in *Pongamia pinnata* are demethoxykanugin, gamatay, glabrin, glabrosaponin, kanjone, kaempferol, karangin, kanugin, quercitin, pinnatin, neoglabin, pongamol, pongapin,  $\beta$ -sitosterol, saponin, and tannin [17–18]. Figure 1 shows chemical structures of active phytoconstituents of *Pongamia pinnata*, which are responsible for its contraceptive activity [19–21].

Our current study evaluated the synthesis and characterisation of AgNPs synthesised from methanolic leaf extract of *Pongamia pinnata* and their contraceptive effect on male rats.

## Material and Methods

Fresh leaves of *Pongamia pinnata* were collected in the Department of Zoology, University of Rajasthan, Jaipur, and authenticated at the Department of Botany with herbarium number RUBL21227. After that, the leaves were washed thoroughly, shade-dried, and converted into fine powder. Silver nitrate ( $\text{AgNO}_3$ ) of analytical reagent grade (AR grade), with CAS Number 7761–88–8, and methanol of Thermo Fisher Scientific (CAS number 67–56–1) were acquired from Rankem India.

# Preparation of leaf extract and silver nanoparticles

50 g of leaf powder was taken and mixed with methanol and double-distilled water in a 1:1 ratio. The mixture was Soxhlet extracted for 8x3 hrs, and then it was filtered with Whatman filter paper number 1. Filtered extract was dried in an incubator at room temperature [22] (Fig. 2). For silver nanoparticle synthesis, 1mM AgNO<sub>3</sub> solution was mixed with plant extract solution and kept at a temperature of nearly 45 ± 5°C till its colour changed from transparent to dark brown. This suggests that the interaction of fresh leaf extract from *Pongamia pinnata* with silver metal ions may have produced the silver nanoparticles. No colour changes were seen when silver nitrate was not added to the control. It validates that there are silver nanoparticles present in the reaction mixture where the colour shifts occur.

## Characterization of Silver Nanoparticles

UV–vis spectrophotometry assessed the reaction solution's absorption spectra after the silver nanoparticles were produced at various intervals (Fig. 3). A centrifuge was used to separate the reaction mixture. A hot air oven was used to gather and dry the pellets. SEM was used to describe the shape and size of the air-dried silver nanoparticles. SEM was done by using **Nova NanoSEM450** at 15.00 kv and high vacuum mode at a line resolution of at 400 nm. EDAX analysis determined whether elemental silver was present in the solution combination. EDAX was done by using **BRUKER** energy dispersive X-Ray Analyzer at Malviya National Institute of Technology, Jaipur. A crystal monochromator using wavelengths of 0.1541 nm in a 2θ range from 5° to 90° was used for XRD (Diffractometer system = XPERT-PRO, PANalytical) equipped with Cu-Kα radiation to further characterize AgNPs. Infrared spectroscopy (IR) is used to identify important functional groups, and biomolecules that are specifically bound to the produced AgNPs by the electrostatic attraction of free amine groups, cysteine residues, or carboxyl groups are described.

## Animals and treatment

Male Wistar rats weighing 150–200 grams and aged 12–16 weeks were acquired from the LUVAS, Hisar Animal House. They were housed in polycarbonate cages with woodchip bedding, fed a typical pellet diet and tap water, and kept at room temperature (22–24°C) with a 12:12 light–dark cycle. Two weeks before the rats were used in the experiment, they were acclimated to the surroundings. The IAEC Animal Ethics Committee's requirements were adhered to in the conduct of this investigation. The Department of Zoology, University of Rajasthan, Jaipur, granted permission from the IAEC (Institutional Animal Ethical Committee) to conduct all animal experiments following the WMA Statement on Animal Use in Biomedical Research, the EU Directive 2010/63/EU, and institutional guidelines approved by the IAEC, guaranteeing adherence to ethical principles including the 3Rs, with proposal number **UDZ/IAEC/2023-1/02**. Five groups of eight rats each were randomly assigned. Each experimental group consisted of eight animals. For simple identification, each group's animals were marked with picric acid on the head, abdomen, tail, back, left upper leg, and left hind leg. They then received the following therapy (Table 1)

Table 1

Animals included in the experiment are divided into treatment groups and given varying dosages of AgNPs of *Pongamia pinnata* for 60 days.

| Treatment groups | Treatment                                             | Duration | Recovery time |
|------------------|-------------------------------------------------------|----------|---------------|
| Group-I          | Distilled water                                       | 60 days  | -             |
| Group-II         | 10 mg/kg Body weight AgNPs of <i>Pongamia pinnata</i> | 60 days  | -             |
| Group-III        | 20 mg/kg Body weight AgNPs of <i>Pongamia pinnata</i> | 60 days  | -             |
| Group-IV         | 20 mg/kg Body weight AgNPs of <i>Pongamia pinnata</i> | 60 days  | -             |
| Group-V          | 30 mg/kg Body weight AgNPs of <i>Pongamia pinnata</i> | 60 days  | 30 days       |
|                  | 20 mg/kg Body weight AgNPs of <i>Pongamia pinnata</i> |          |               |

## Fertility Test

Each rat's fecundity was evaluated both prior to and during therapy. For five days, male and female rats were mated in a 1:2 male: female ratio to assess fertility [23].

$$\text{Male Fertility Index} = \frac{\text{Number of males impregnating female}}{\text{Number of males cohabited}} \times 100 \text{ [24]}$$

## Tissue Biochemistry

For biochemical examination, the testes, ventral prostate, vas deferens, seminal vesicles, and epididymides were meticulously removed, washed in saline solution, and stored at -20°C. Total protein in male reproductive tissues like testis, ventral prostate, seminal vesicles, and epididymides was evaluated [25].

## Histopathological Studies

Using conventional histology methods, epididymis was preserved in Bouin's solution, dried in graded ethanol, cleaned in xylene, and embedded in paraffin wax. After the slices were cut at 6 µ and stained with Harris's hematoxylin and eosin (H&E) stain, they were examined under a light microscope. Using a light microscope set to 100X magnification, portions of the epididymis were examined and photographed to examine its histological structure.

## Transmission Electron Microscopic Analysis

Testis tissues were sliced into tiny 1 mm<sup>3</sup> pieces and fixed right away in a modified version of Karnovsky's fixative (4% glutaraldehyde + 4% paraformaldehyde + 0.2% picric acid + 0.02% calcium

chloride + 0.2 M cacodylate buffer). They were then dehydrated in graded acetone solutions and embedded in an aldite resin. Rinsing, post-fixation, dehydration, and infiltration were all done in the KOS microwave tissue processor. With a glass knife and a Leica Ultracut R Ultramicrotome, semi-thin pieces were cut to a thickness of 1  $\mu\text{m}$ . At the Sophisticated Analytical Instrument Facility, All India Institute of Medical Sciences (SAIF-AIIMS), New Delhi, the slices were examined and captured on camera using the Talos HR-TEM (120 kv).

## Statistical Analysis

Tukey's one-way ANOVA for independent samples was utilized for comparisons between more than two groups to examine all AgNP treatment outcomes across control and treatment groups. When comparing the means of three or more groups, this test is frequently employed to find a significant difference. Data visualization and statistical analysis were conducted using Origin 8 software and Microsoft Excel 2016. The results were displayed as mean  $\pm$  standard error (mean  $\pm$  SE), with a P value of  $< 0.05$  deemed significant when  $n = 8$  ( $n$  = number of samples in each group).

## Results

### Characterization by UV-VIS spectrophotometer

The main study of silver nanoparticle synthesis was conducted using UV-Vis spectrophotometric analysis. There is a noticeable shift in shade in the mixture of AgNPs and plant extract. Absorbance peak was observed for AgNPs of *Pongamia pinnata* and with plant extract only. The AgNPs of *Pongamia pinnata*'s absorbance peak was observed at 424 nm (Fig. 4A). Time-based data were also collected for 15 minutes, 30 minutes, 45 minutes, and 60 minutes, and their absorbance peaks were also observed (Fig. 4B)

### Fourier Transform Infra-Red (FTIR)

To find the bioactive substances causing the decrease of silver ions, FTIR spectroscopic analysis was done. FTIR spectrum of the plant extract and AgPs of *Pongamia pinnata* are shown in Fig. 5. Identifiable peaks in the *Pongamia pinnata* AgNPs (Fig. 5A) were found at 3431.86, 2931.4, 2351.8, 1635.98, 1385.8, 1019.5, and 723.42  $\text{cm}^{-1}$ . The existence of carboxylic acids (O-H group) was responsible for the band peak at 2931  $\text{cm}^{-1}$ . The presence of C-H from methyl or methylene groups was responsible for the band peak at 2351  $\text{cm}^{-1}$ . The presence of aromatic rings at 1635  $\text{cm}^{-1}$  was attributed to the binding of C-O functional groups, which suggested the presence of tannins on the surface of Ag-core particles. The findings demonstrate that tannins, in particular, prevent AgNPs from aggregating and maintain the long-term stability of the nanoparticles. The aromatic amine group C-N peak at 1385  $\text{cm}^{-1}$  would indicate the C = H bending vibrations, while the carboxyl group of AgNPs could be responsible for the peak at 1019  $\text{cm}^{-1}$ . In (Fig. 5B), FTIR spectra of plant extract of *Pongamia pinnata* show the absorption of peaks 2850–3300  $\text{cm}^{-1}$  for C-H group, 1000–1300  $\text{cm}^{-1}$  for C-O group, and 2500–3300  $\text{cm}^{-1}$  for O-H groups.

# Powder X-Ray Diffractometer (PXRD)

The crystalline state of the AgNPs of *Pongamia pinnata* is ascertained by PXRD. The nanoparticles' size was determined using the Debye-Scherrer equation, which reads

$$D = k\lambda/\beta\cos\theta$$

Where K is Scherrer's constant (0.9),  $\lambda$  is the wavelength of the X-ray source (0.154nm),  $\beta$  is FWHM, and  $\theta$  is Bragg's angle.

The  $2\theta$  peaks of *Pongamia pinnata* were seen to have a Bragg reflection between 27.845° and 77.01304°. This reflection was associated with the (100), (111), (231), and (311) flat lattice. This may be ascribed to the face-centered cubic crystal structure of the silver, which was manufactured using **Origin Pro 8**. The crystallite size was calculated by using Scherrer's equation, and the average size was found to be 39.241 nm (Table 2 & Fig. 6).

| Table 2<br>Crystalite size of AgNPs of <i>Pongamia pinnata</i> leaf extract |                     |     |                     |        |
|-----------------------------------------------------------------------------|---------------------|-----|---------------------|--------|
| SAMPLE                                                                      | $2\theta(^{\circ})$ | hkl | FWHM ( $^{\circ}$ ) | D (nm) |
| AgNPs                                                                       | 27.845              | 100 | 0.1181              | 69.276 |
|                                                                             | 32.225              | 122 | 0.2362              | 34.995 |
|                                                                             | 38.075              | 111 | 0.4133              | 20.325 |
|                                                                             | 46.295              | 231 | 0.2066              | 41.802 |
|                                                                             | 54.815              | 142 | 0.2066              | 43.297 |
|                                                                             | 57.515              | 241 | 0.2952              | 30.685 |
|                                                                             | 76.715              | 311 | 0.2952              | 34.305 |
| Mean                                                                        |                     |     |                     | 39.241 |

# Field Emission- Scanning Electron Microscope (FE-SEM) & Energy Dispersive X-Ray Analysis (EDAX)

One of the most effective instruments for determining the shape of the produced nanoparticles is a scanning electron microscope. The fresh leaf extract from *Pongamia pinnata* produces silver nanoparticles that are primarily spherical in form (scale bar 400 nm). The development of large-sized nanoparticles is the outcome of the nanoparticles' aggregation. Furthermore, the enhanced aggregation of the biosynthesized AgNPs may have resulted from the dehydration required to get the samples ready for SEM examination. This report and the UV-vis spectra report complement each other nicely. Silver

nanoparticles of various sizes, spherical and irregularly shaped, were well distributed in the SEM image (Fig. 7). According to SEM measurements, AgNPs of *Pongamia pinnata* are between 25 to 50 nm in size. The creation of metallic silver nanoparticles in the reaction mixture was verified by elemental analysis. Figure 8 displays the EDAX analysis of the silver nanoparticle formation mediated by fresh leaf extract from *Pongamia pinnata*. At 3 keV, the EDAX analysis revealed a strong signal, indicating the presence of elemental silver. In EDX analysis, it showed Ag 44.49%, Si 39.68%, Na 7.73%, Cl 5.40%, and Mg 3.50%. (Fig. 8).

**Impacts of Pongamia pinnata AgNP therapy on the fertility index of male rats**

After the last dosing to male rats of AgNPs of *Pongamia pinnata*, male rats were kept with female rats for 5 days for post-mating. And its data is shown in Table 3. The number of pups delivered in different groups of individual animals is shown in Fig. 9.

Table 3  
Fertility index of treated male rats with AgNPs of *Pongamia pinnata*

| GROUP                                     | Number of mated males | Number of mated females | Pregnant female | Fertility% | Number of pups delivered |
|-------------------------------------------|-----------------------|-------------------------|-----------------|------------|--------------------------|
| GROUP-I<br>(Control group)                | 08                    | 16                      | 16              | 100% (+)   | 40                       |
| GROUP-II<br>(10 mg/kg)                    | 08                    | 16                      | 12              | 75% (-)    | 16                       |
| GROUP-III<br>(20 mg/kg)                   | 08                    | 16                      | 08              | 50% (-)    | 10                       |
| GROUP-IV<br>(30 mg/kg)                    | 08                    | 16                      | 06              | 37.50% (-) | 03                       |
| GROUP-V<br>(20 mg/kg)<br>(Recovery group) | 08                    | 16                      | 14              | 87.50% (-) | 22                       |

**Tissue Biochemistry**

Other treated groups with a dose of AgNPs of *Pongamia pinnata* for 60 days showed a decrease in protein concentration compared to the control group. Recovery group V showed a non-significant change in protein amount compared to control group, analysed in different reproductive tissues like testes, seminal vesicles, epididymides, and ventral prostate (Fig. 8).

**Histopathological Analysis**

The histology of cauda epididymis of rat in control group showed pseudostratified columnar epithelium with principal cells that possess long stereocilia, aiding in fluid absorption and sperm concentration. The cauda architecture had degenerative alterations in animals from the lower dose group. There were fewer trash particles and spermatozoa in the cauda epididymis lumen. The caudal epithelium of the epididymis showed notable degeneration in the intermediate and higher dose group animals, and the lumen showed no spermatozoa count. These animals' cauda epididymis was noticeably altered. Following a 30-day break from treatment, the recovery group's cauda epididymis histoarchitecture showed a notable improvement (Fig. 9).

## Transmission Electron Microscopy

The testicular architecture of the control rats (Group I) was normal, as revealed by ultrastructural analysis. It had a thick basal lamina, myoid cells encircling it, and spermatogonia grouped consistently. A round or oval nucleus, coarsely granular nucleoplasm, sparse cytoplasm, and granular ribonucleic protein particles were characteristics of the spermatogonial cell. The tubular limiting membrane was parallel to their nuclei, which were in close contact with the basal lamina. Compared to the control groups, the seminiferous tubules in the group treated with AgNPs of *Pongamia pinnata* were thicker with fibrous connective tissue. It revealed a severely damaged irregular tubular basement membrane that extended finger-like into the cytoplasm. In germ cells, group III & IV showed cellular change and apoptosis. It was clear that notable gaps were separating the spermatogonia cells from the basal lamina and nearby cells. There was a disruption in the spermatogonium's nuclear envelope.

Nanoparticles were clearly accumulating close to the basement membrane. The treated animals' spermatogonial cells showed clumped chromatin, degraded cytoplasm, and a nucleus. In recovery group V it showed regeneration of germinal epithelium and spermatozoa in lumen of seminiferous tubules (Fig. 10)

## Discussion

In the present study male contraceptive activity of AgNPs of *Pongamia pinnata* was evaluated by using methanolic leaf extract. The findings of the current work discuss the successful synthesis and characterization of silver nanoparticles. UV-Visible spectroscopy confirms the synthesis of AgNPs of *Pongamia pinnata*, and an absorbance peak was observed around 424 nm. A first step in verifying the creation of AgNPs is color identification. It might result from the lowering of AgNO<sub>3</sub> and the stimulation of the Surface Plasmon Resonance (SPR) effect [26]. It was observed that the absorption peak width progressively shrank over time, indicating that the freshly generated AgNPs have a limited size distribution [16]. The peaks at 424 nm, which are produced by *Pongamia pinnata* extracts, show the presence of silver nanoparticles. The reaction mixture's electron surface plasmon resonance caused the peak to rise [10]. The straightforward method of producing AgNPs from plants involves interactions between AgNO<sub>3</sub> and the biomolecule components of plant extracts.

The creation of nanoparticles occurs in three major stages: The synthesis of nanoparticles is initiated by (1) an ion reduction reaction (2) that results in cluster formation (3) Plant biomolecules include hydroxyl groups (OH), which stabilize and convert silver ions ( $\text{Ag}^+$ ) to silver ( $\text{Ag}^0$ ). Silver nuclei are created when it is further reduced to  $\text{Ag}^+$ , resulting in AgNPs [27]. The existence of aromatic amines, amide groups, phenolic groups, and secondary alcohols was confirmed by FTIR spectra, and these substances may function as reducing agents during the creation of AgNPs [28]. The Scherrer equation was applied to get the average crystallite size [29–30; 7]. The crystalline character of the produced nanoparticles is confirmed by the strong peaks, which are in good agreement with past studies [31]. The synthesis and stabilization of plant metabolites promote the creation of silver nanoparticles at the nanoscale range in SEM analysis. Comparable outcomes were also documented when silver nanoparticles were synthesized using additional phytochemical components [32]. Silver ( $\text{Ag}^+$ ) atoms were shown to be responsible for the largest percentage of the peak intensity in the nanoparticle's EDX examination. When silver particles were examined using EDX, a strong signal was found in the 2.8–3.4 keV range [33]. In present study, fertility reduction was observed as dose level of treatment increased; hence, fertility indices of treated rats were reduced [34]. Treatment with a dose of AgNPs of *Pongamia pinnata* also caused a reduction in body weight and reproductive organs. It also caused a significant reduction in testosterone level and tissue biochemical parameters, which include glycogen, sialic acid, cholesterol, and fructose. Sperm motility and sperm density were also reduced drastically, supporting the current work findings for male contraception [17]. Total protein of reproductive tissues like testes, seminal vesicles, ventral prostate, and epididymides was analysed, and its significant reduction was observed. Lack of sufficient protein has profound impacts on the maturation of epididymal sperm and spermatogenesis. The Sertoli cell is known to regulate and support spermatogenesis through a variety of protein factors. Spermatogenesis would be significantly delayed by a lack of sufficient protein. Reduced testicular weight and delayed germ cell maturation are the results of protein deficiencies, according to studies [35]. Cauda epididymis was stained with H & E stain. And its transverse section was observed under a light microscope. Rats given AgNPs exhibited dose-dependent degeneration of the epithelial lining, loss of stereocilia, vacuolation of the epithelial cells, and a significant decrease in or lack of spermatozoa in the lumen. These histological alterations point to compromised sperm maturation and transport, probably why fertility indices have declined [36]. Histologic alterations in epididymis indicate a disturbance in the intratesticular androgen level because the epididymis is an essential reproductive organ that primarily depends on androgen to maintain its structure and function [15]. The TEM analysis can be used to comprehend testicular physiology, spermatozoa status, and cellular architecture. Additionally, it helps to clarify the roles of the cellular organelles in reproduction [37]. In mammals, spermatogenesis is a highly coordinated and dynamic process that produces spermatozoa continuously. According to our transmission electron microscopy results, the cytoplasm of spermatogonia, primary and secondary spermatocytes, round and elongating spermatids, and Sertoli cells in the experimental group underwent significant cellular changes, indicating changes in these cells' functions [38]. It might be due to that cross-sections of the centre piece of mature sperm were examined under an electron microscope, the mitochondrial sheath swelled, and the cell membrane was uneven. Cytoplasmic vacuoles were visible in certain portions. Since sperm cytoplasm lacks these antioxidative enzymes and the plasma membrane

of spermatozoa contains a large amount of polyunsaturated fatty acids, sperm are especially susceptible to free radical attack, which can cause lipid peroxidation and worsen sperm defects [39]. The basement membrane, which preserves the structural and functional integrity of testicular tissues, also underwent degenerative alterations as a result of nanoparticles. Modified basement membrane structure may cause the testis to become severely impaired in its ability to function [38]. Because nanoparticles damaged or interfered with the functioning of spermatogonial cells, Sertoli cells, and the basement membrane, the number of germ cells eliminated by apoptosis increased. Deformed spermatocytes, spermatids, and detached germ cells may be observed as a result of a significant disturbance in the interaction between Sertoli and germ cells. The seminiferous epithelium's germ cells may have shed as a result of the disturbance in this physical connection [40].

## Conclusion

The present study successfully demonstrates the green synthesis of AgNPs using methanolic leaf extract of *Pongamia pinnata*, highlighting an eco-friendly and cost-effective approach to nanoparticle fabrication. The biosynthesized AgNPs exhibited significant antifertility effects in male rats, as evidenced by marked histopathological alterations observed in epididymal tissues. Transmission Electron Microscopy provided high-resolution imaging that revealed ultrastructural disruptions, including degeneration of spermatogenic cells, vacuolization, and mitochondrial abnormalities, supporting the antifertility potential of the synthesized nanoparticles.

Furthermore, the antifertility assessment revealed that administration of these biosynthesized AgNPs significantly impacted male reproductive parameters in rats. Observed effects included reduced sperm count, motility, and alterations in testicular histoarchitecture, suggesting potential interference with spermatogenesis and hormonal balance. These findings indicate a possible use of *Pongamia pinnata*-derived AgNPs as a male antifertility agent, warranting further exploration into their mechanism of action, long-term safety, and reversibility.

Overall, this research provides a dual insight into both nanobiotechnology and reproductive biology, establishing a foundation for future studies on plant-based nanomaterials in therapeutic and reproductive health contexts.

## Declarations

## Competing interests

The authors declare no competing interests.

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## Author Contribution

PY: Methodology, data analysis, data curation (lead), and writing the original Draft (lead), Editing, Investigation, Revision, Software analysis, and Formal analysis. PCM: Supervision (lead), Investigation, Conceptualization (lead), project administration. NB: Formal analysis, Investigation, and data validation (equal). All authors have made their substantial contribution, approved, and agree to be accountable for all parts of this work.

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## Data Availability

No datasets were generated or analysed during the current study.

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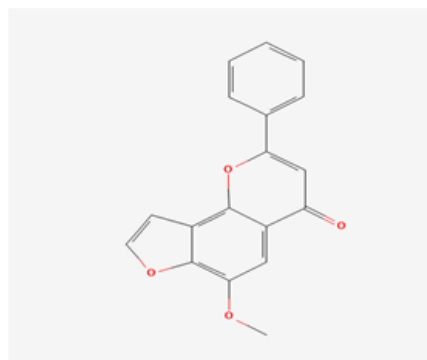
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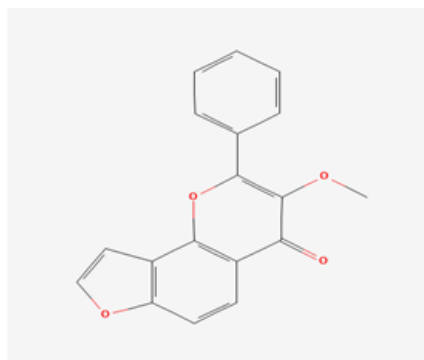
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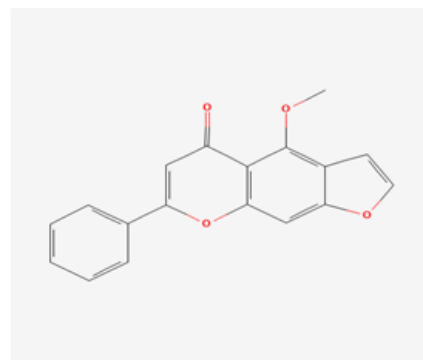
## Figures



**Kanjone**  
[6-methoxy-2-phenylfuro[2,3-b]chromen-4-one]



**Karanjin**  
[3-methoxy-2-phenylfuro[2,3-b]chromen-4-one]



**Pinnatin**  
[4-methoxy-7-phenylfuro[3,2-g]chromen-5-one]

**Figure 1**

Chemical structure of active phytoconstituents of *Pongamia pinnata*

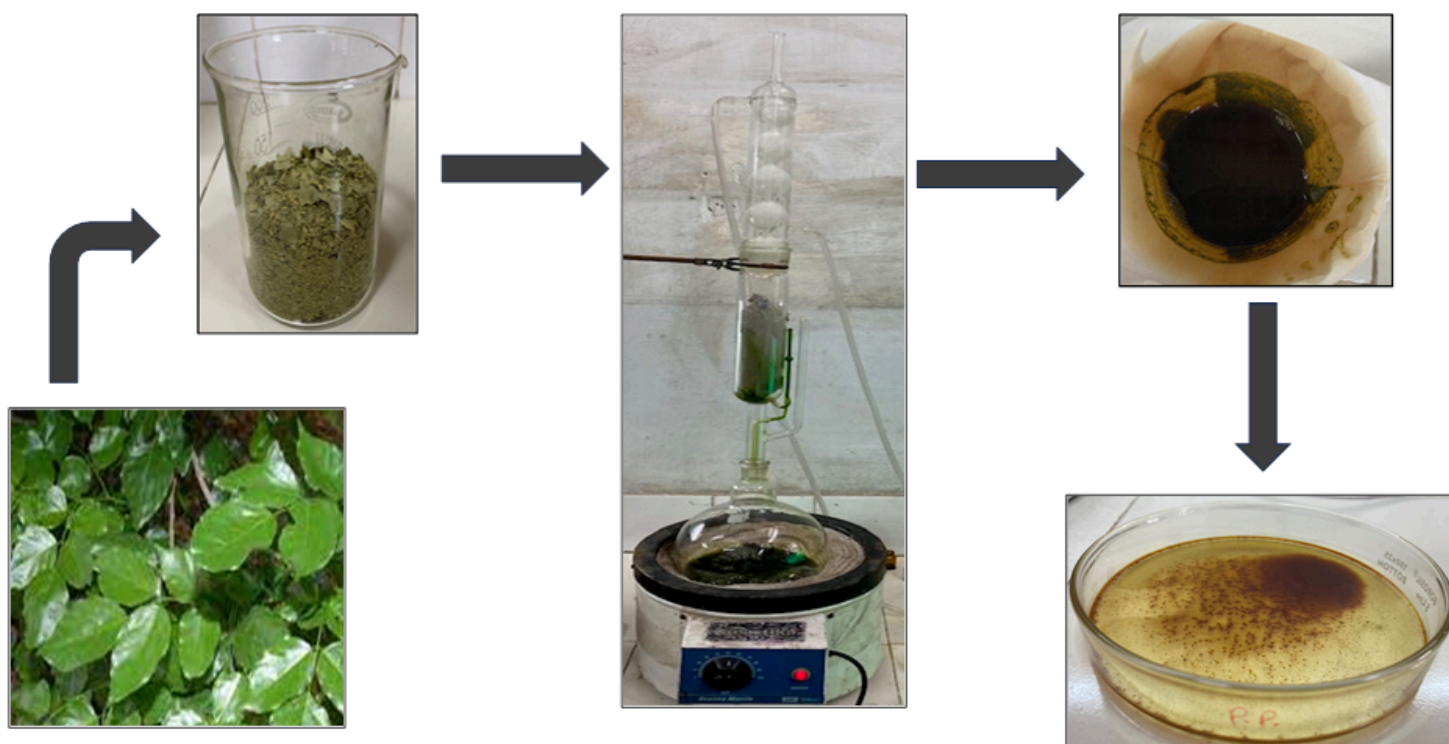


**Figure 2**

Schematic presentation of the collection, synthesis, and extraction of leaf material of *Pongamia pinata*

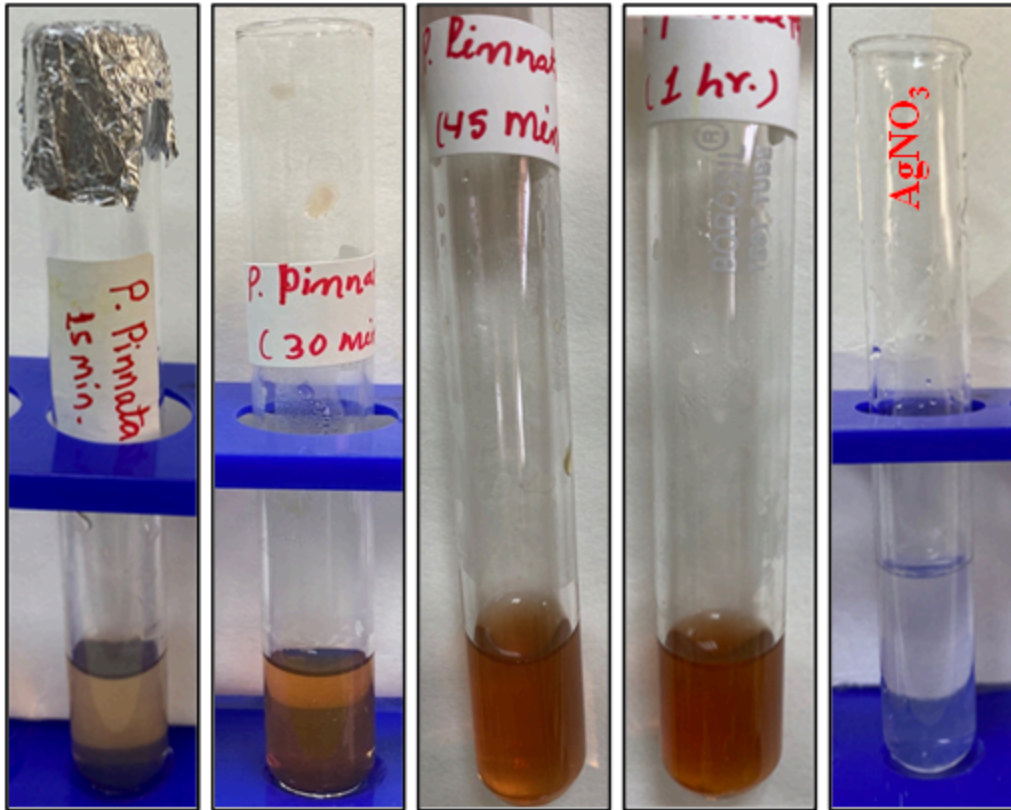


**Figure 3**

Time-based color changes in *Pongamia pinnata* AgNPs and  $\text{AgNO}_3$  solution

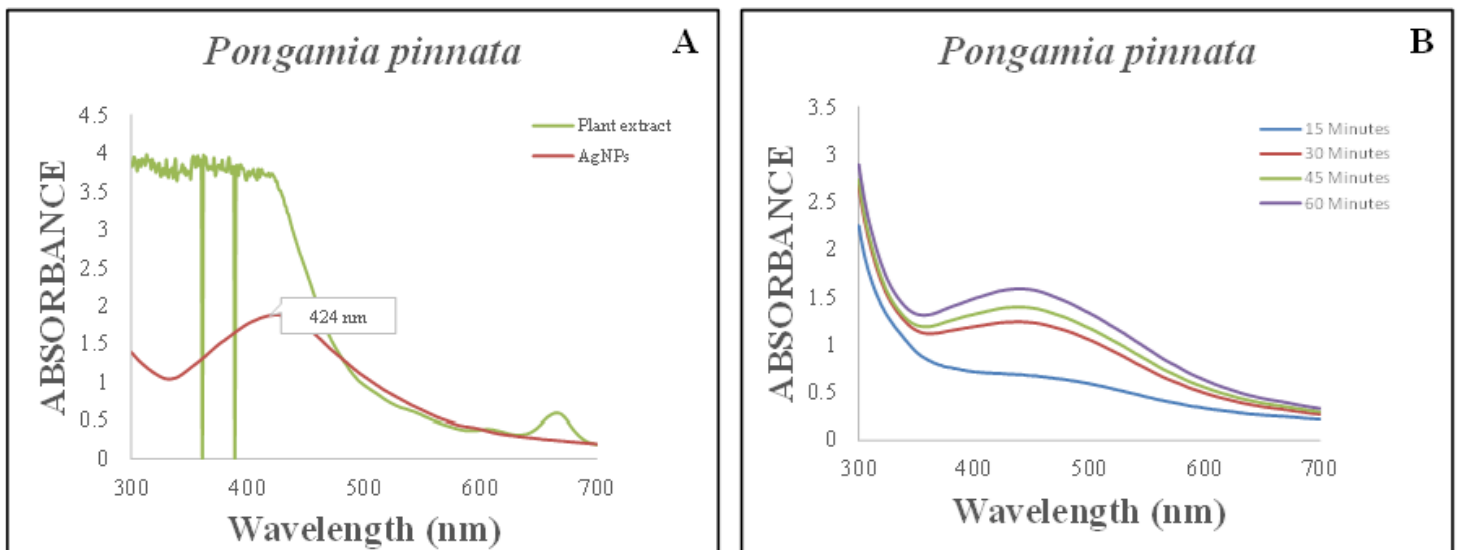


**Figure 4**

UV-VIS spectroscopy of AgNPs of *Pongamia pinnata* (A) Comparative with plant extract and AgNPs of *Pongamia pinnata* (B) Time based absorbance of AgNPs of *Pongamia pinnata*

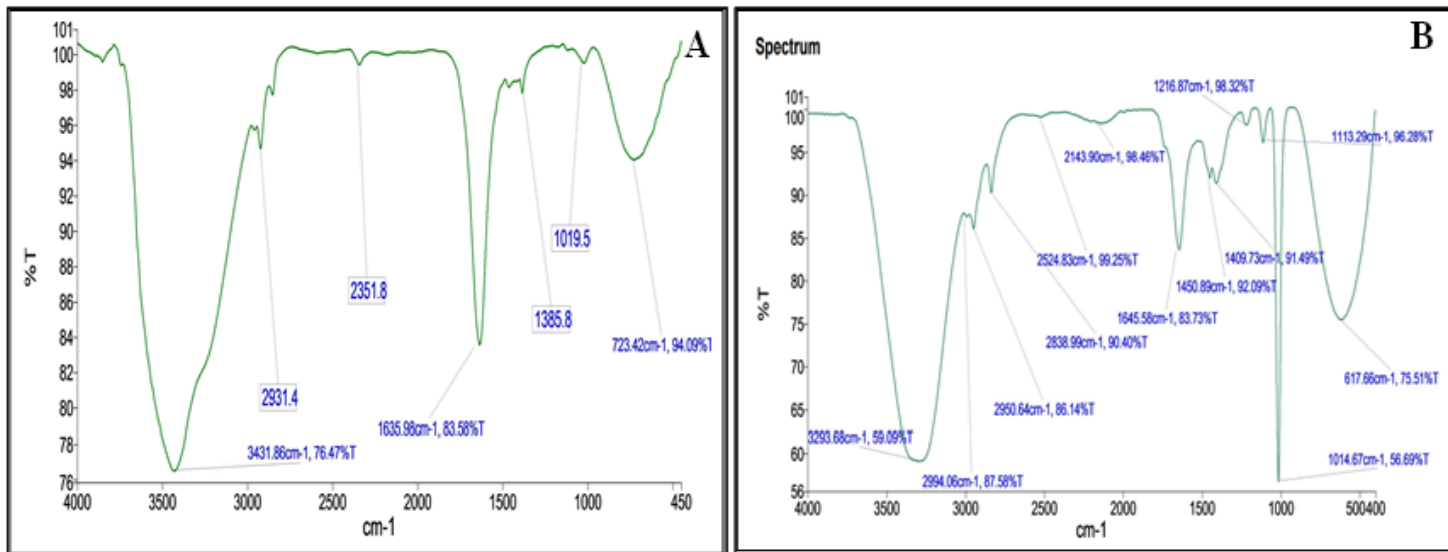


Figure 5

FTIR spectrum of (A) AgNPs of *Pongamia pinnata*, (B) Leaf extract of *Pongamia pinnata*

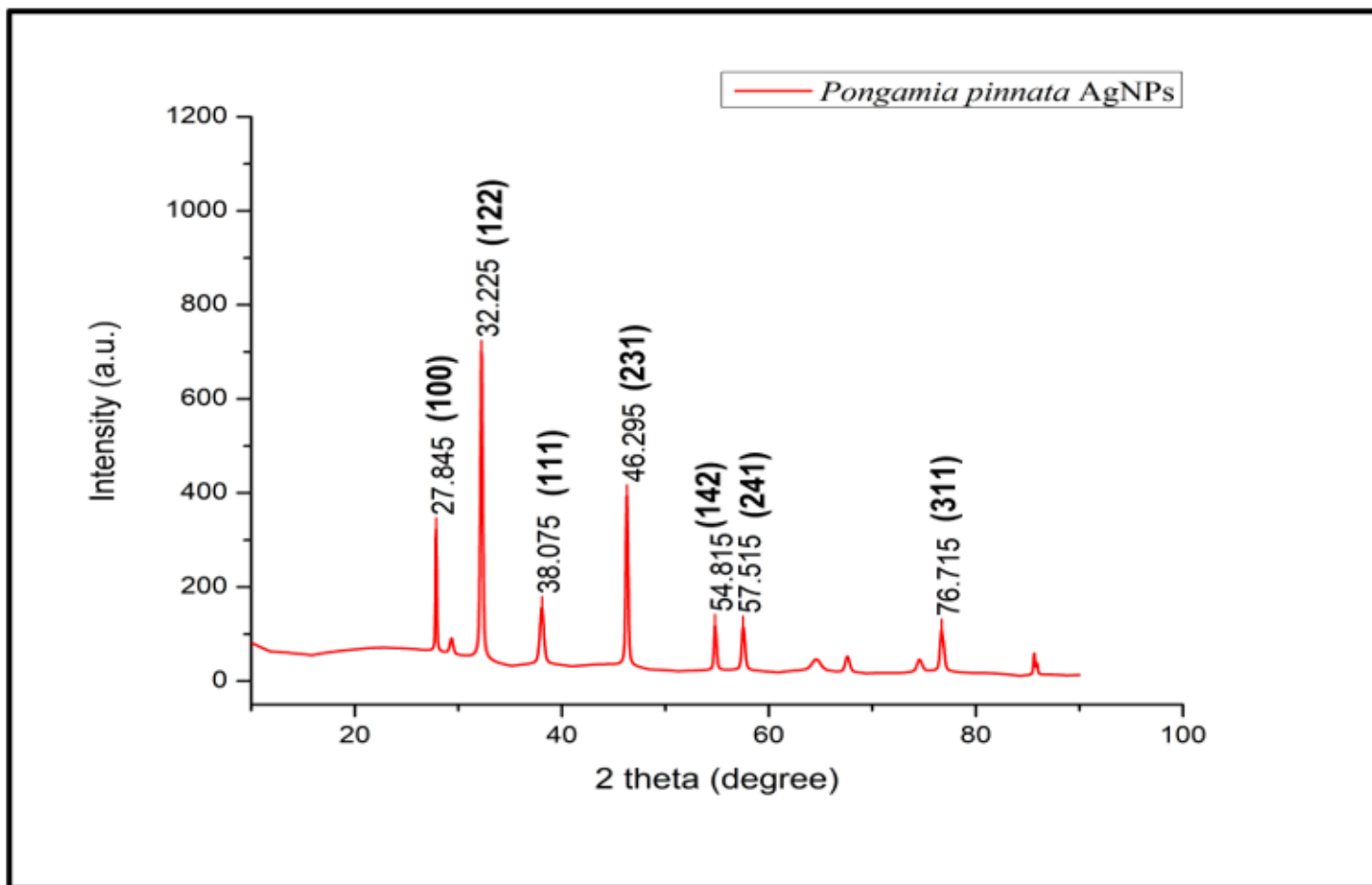


Figure 6

XRD pattern of AgNPs of *Pongamia pinnata* leaf extract

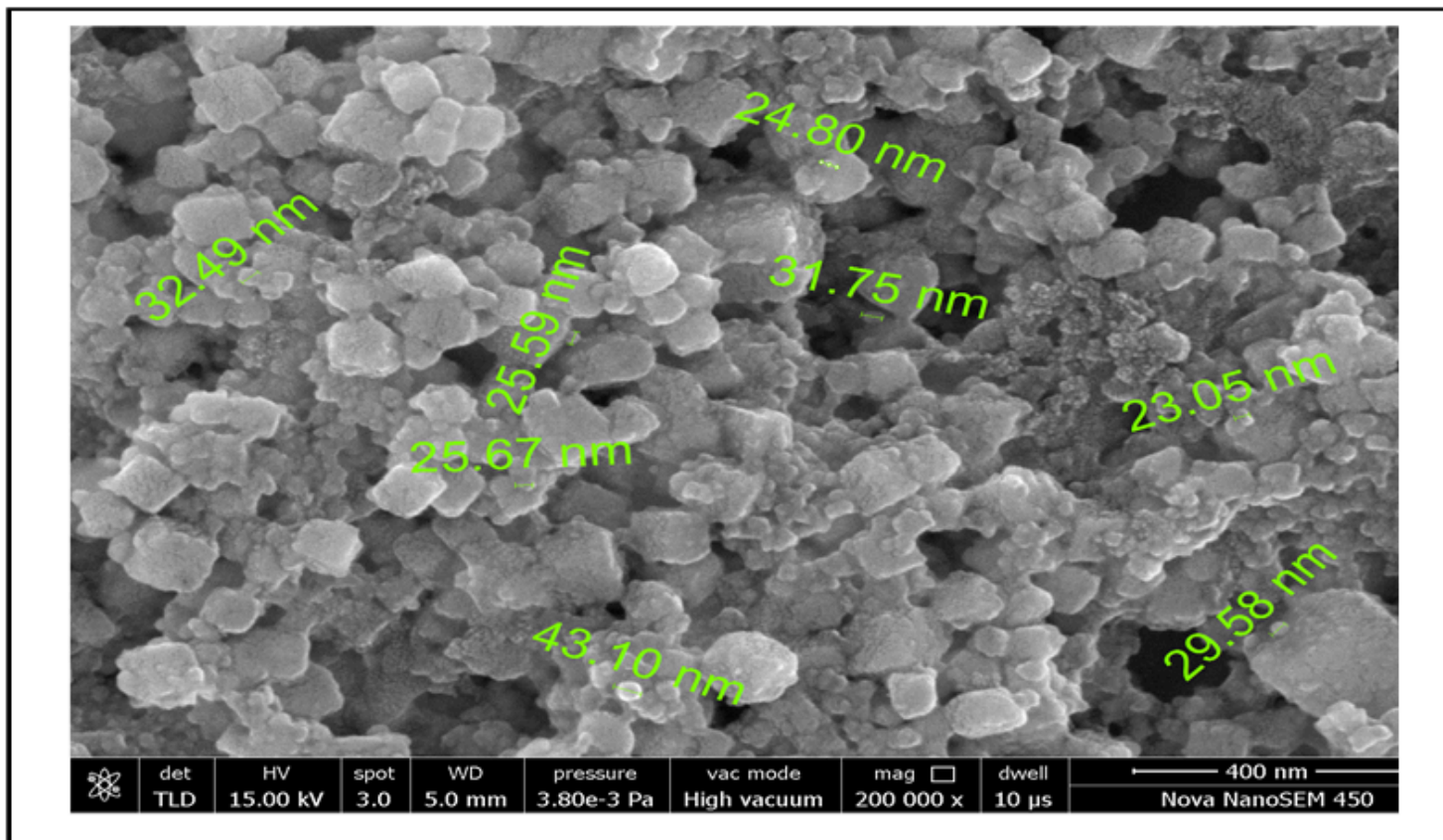


**Figure 7**

SEM image of AgNPs of *Pongamia pinnata*

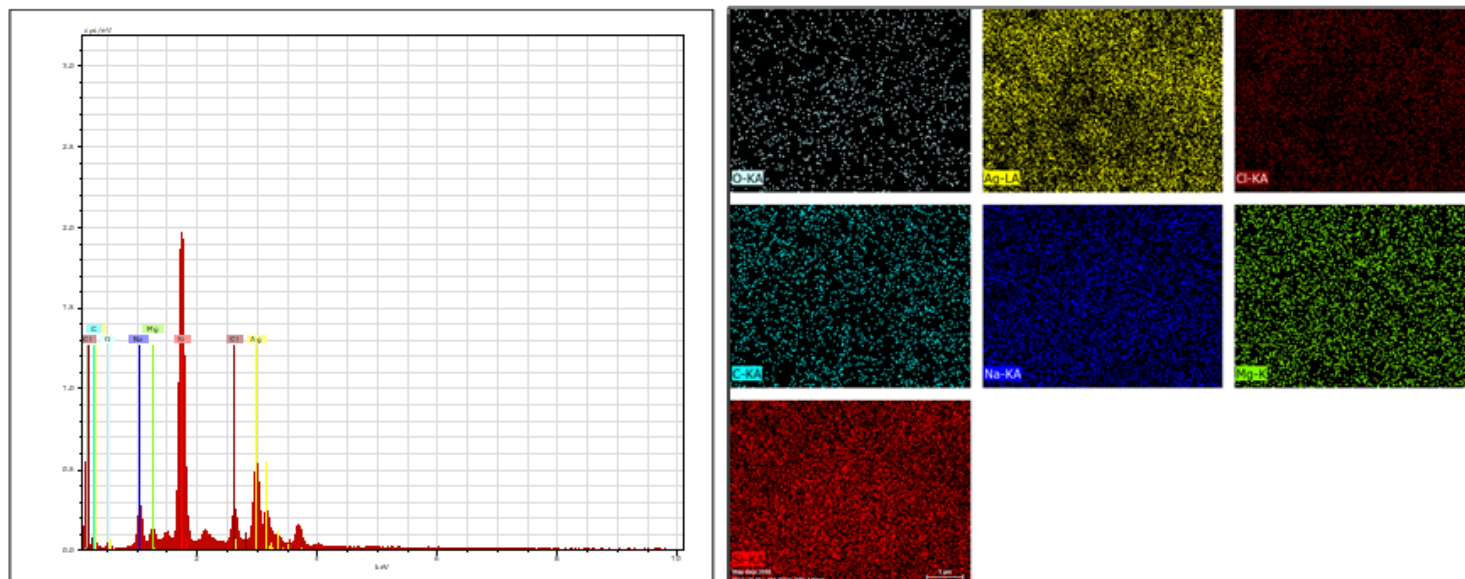


**Figure 8**

EDAX analysis of AgNPs of *Pongamia pinnata* leaf extract and its elemental details

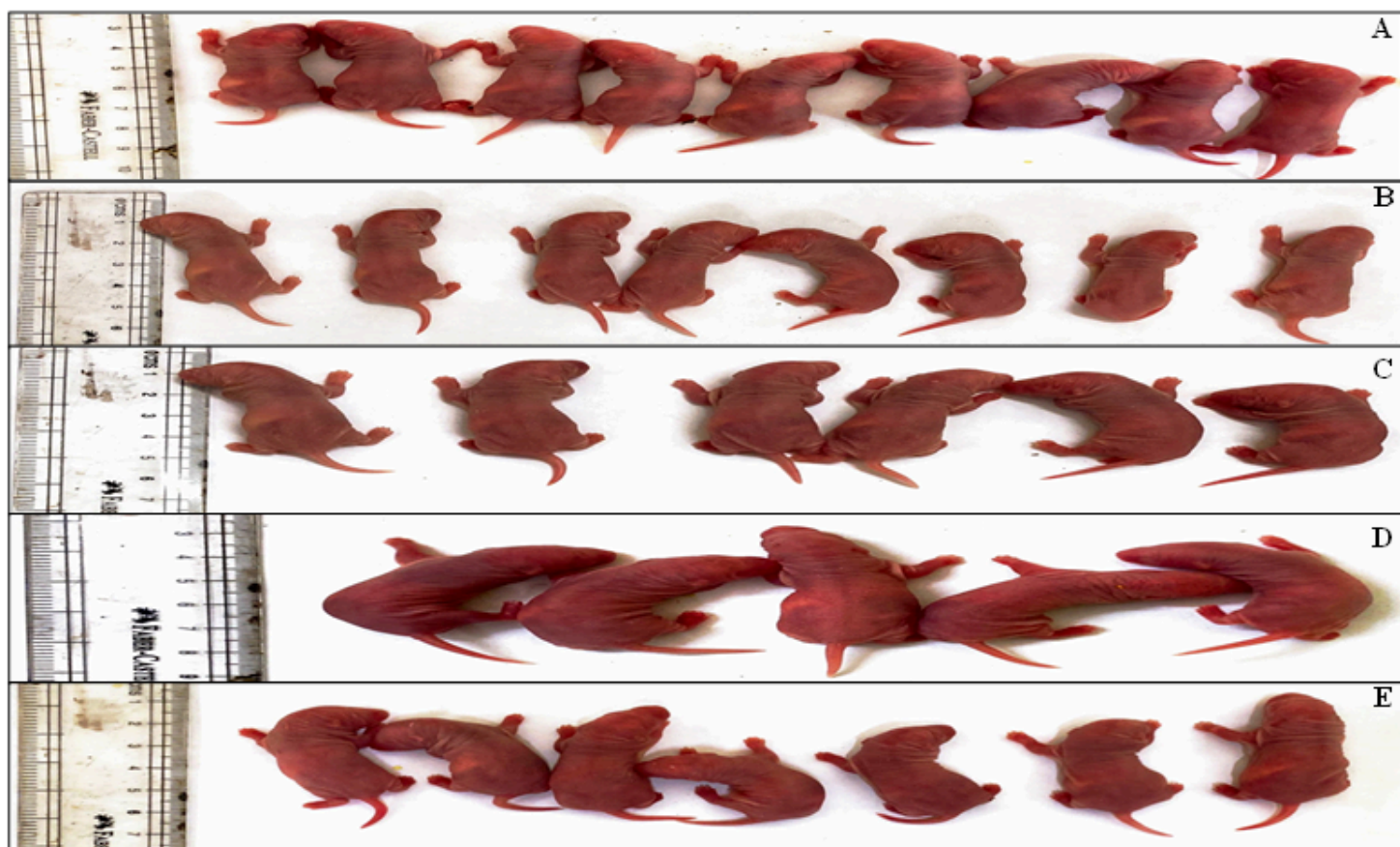


**Figure 9**

Figure 7 Post-mating fertility assessment of treated rats with AgNPs of *Pongamia pinnata* (A) Animal of control group-I treated with distilled water (B) Rats of group-II treated with a dose of AgNPs of 10 mg/kg (C) Group-III received a dose of 20 mg/kg of AgNPs (D) Group-IV received a high dose of AgNPs of 30 mg/kg (E) Recovery group-V received a middle dose of 20 mg/kg for 60 days and kept without any treatment for 30 days

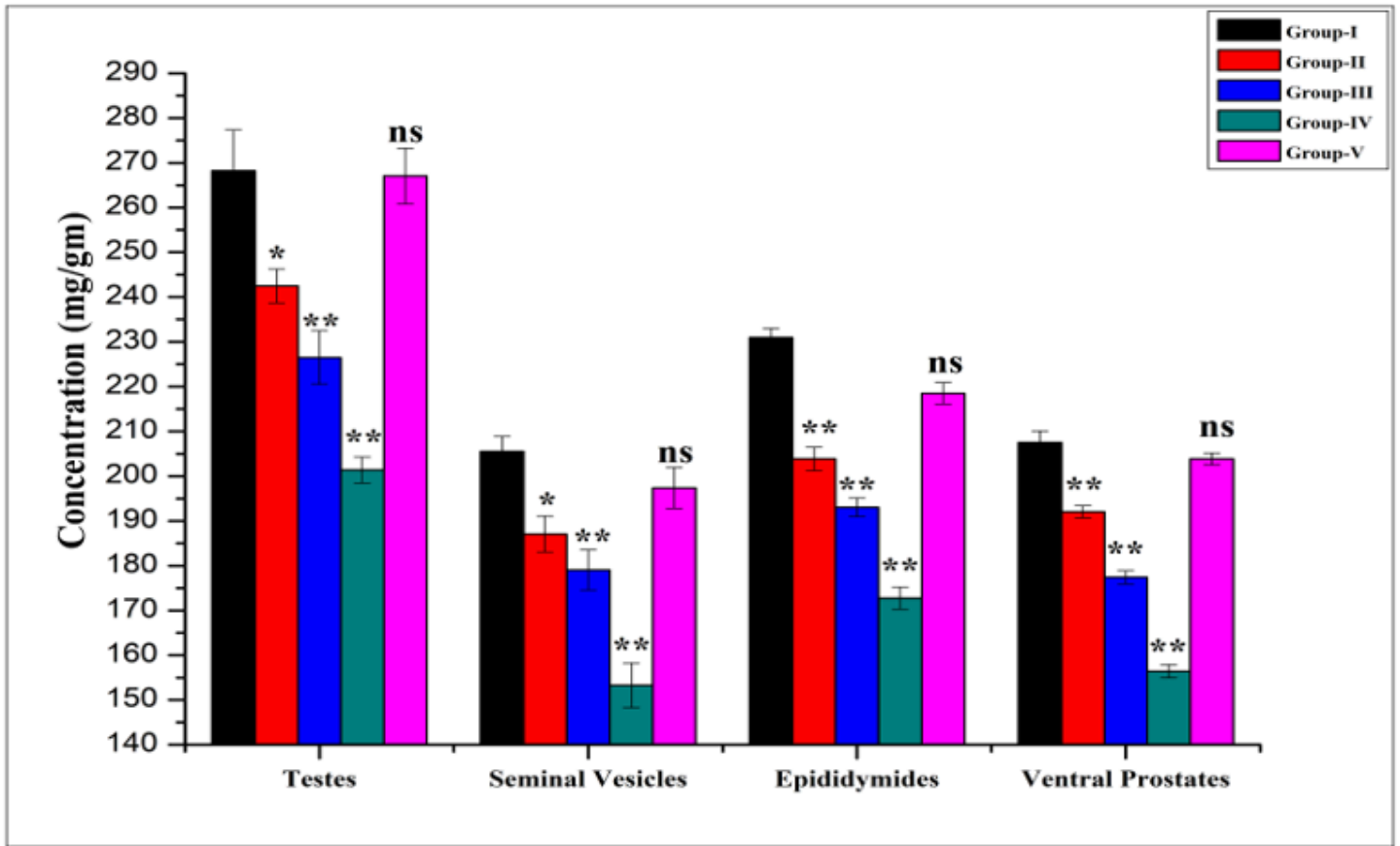


**Figure 10**

Figure 8 Changes in protein concentration of different reproductive tissues like testes, seminal vesicles, ventral prostate, and epididymides of male rats treated with different concentrations of AgNPs of *Pongamia pinnata* for 60 days, and group V showed recovery in protein amount when compared to control group.

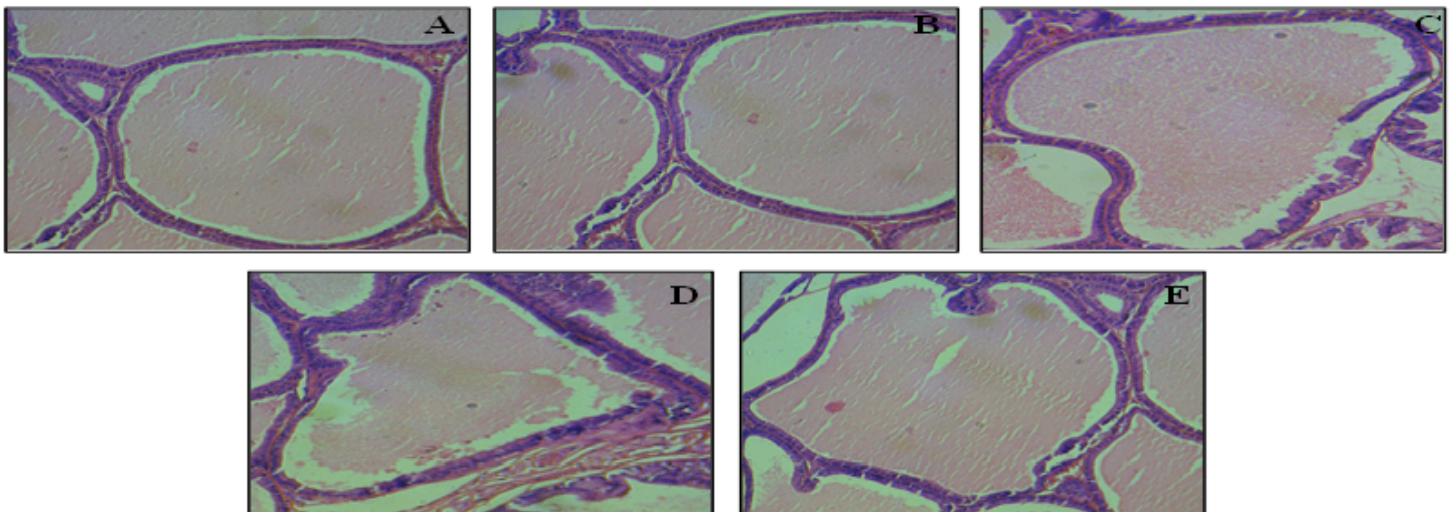


**Figure 11**

Figure 9 Histological analysis of epididymal tissue after administration of AgNPs produced by *Pongamia pinnata*. Sections stained with H&E (100 X) reveal: (A) Group I: control; showing normal epithelial lining and densely packed luminal spermatozoa; (B) Group II: 10 mg/kg AgNPs; exhibiting mild epithelial disorganization and a slight decrease in luminal sperm density; (C) Group III: 20 mg/kg AgNPs; a noticeable decrease in sperm content with signs of epithelial atrophy; (D) Group IV: 30 mg/kg AgNPs; severe spermatotoxic effect; (E) Group V: recovery group; restored epithelial integrity and reappearance of spermatozoa, suggesting partial reversibility of the antifertility effect after treatment cessation

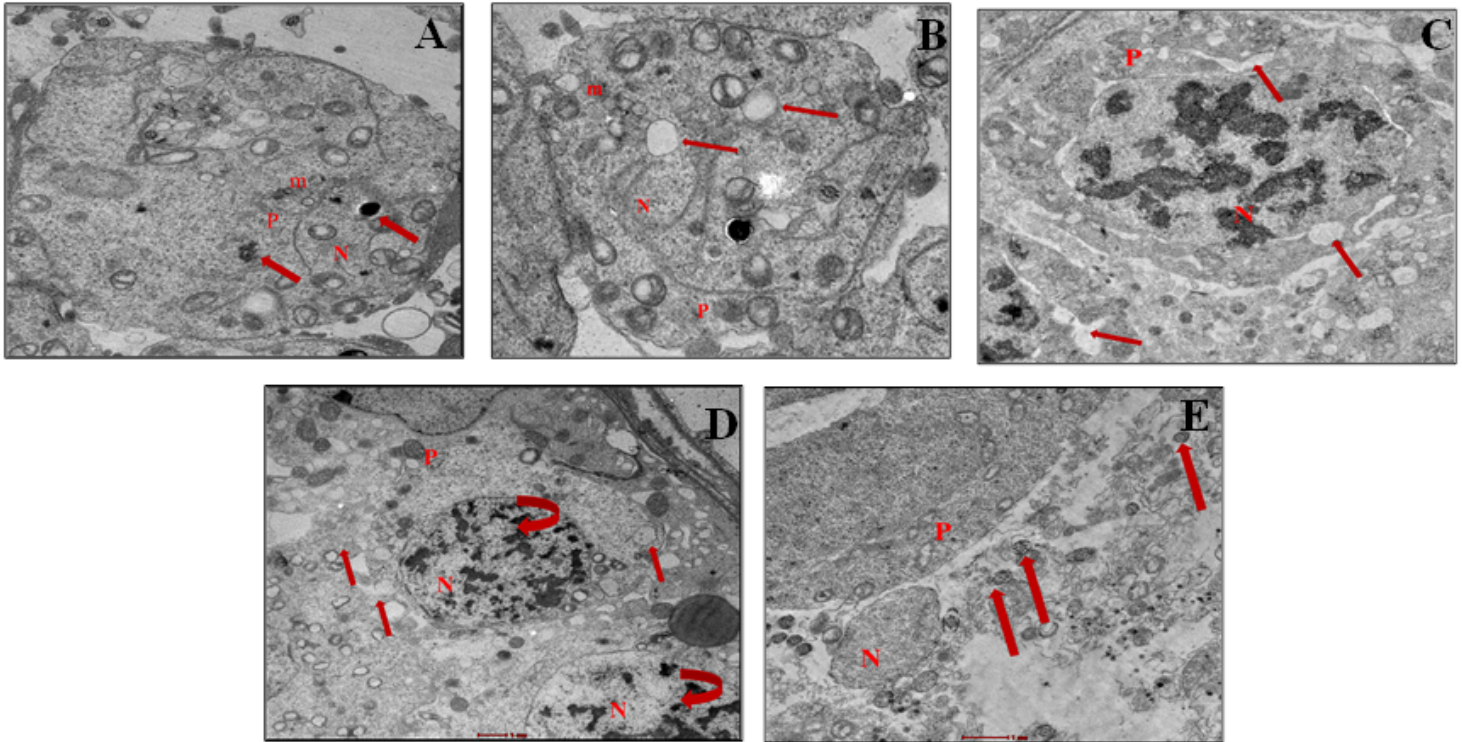


**Figure 12**

Figure 10 TEM of rat's testis (A) control group which shows intact germinal epithelium with mature spermatozoa on lumen of seminiferous tubules (B) Group-II treated with a dose of 10mg/kg of AgNPs of *Pongamia pinnata* for 60 days showed slight degenerative changes in its germinal epithelium, (C) group-III treated with a dose of 20 mg/kg AgNPs of *Pongamia pinnata* for 60 days exhibited damaged and irregular basement membrane with a few spermatozoa in its lumen (D) Group-Iv with highest dose of 30 mg/kg of AgNPs of *Pongamia pinnata* for 60 days showed severe damage in its basement membrane and absence of spermatozoa in lumen with clumped bodies, (E) Group-V recovery group showed some regenerative changes in its germinal epithelium which received a middle dose of 20 mg/kg of AgNPs of *Pongamia pinnata* for 60 days and kept for 30 days after withdrawal of treatment. [N- nucleus, m- mitochondria, P- Primary spermatocyte, curved arrow- coarse clumps of heterochromatin, arrow- intracellular vacuolations]