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Original Article

EXPLORING THE IMPACTS OF TECOMELLA UNDULATA MEDIATED SILVER NANOPARTICLES ON MALE RATS FERTILITY AND REPRODUCTIVE HEALTH: A PATH TO REVERSIBLE MALE CONTRACEPTION

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ABSTRACT

Objective: The present study was designed to evaluate the antifertility effects of AgNPs synthesized using *Tecomella undulata* methanolic leaf extract in male albino Wistar rats.

Methods: The AgNPs were synthesized and characterized by UV-visible spectrophotometry, FTIR, XRD, SEM-EDX and ZETA potential. The antifertility effects of AgNPs were evaluated, dividing 40 male rats into five treatment Groups, G-I treated with sterile distilled water and G-II, G-III and G-IV with 10, 20 and 30 mg/kg. B. wt/D of *T. undulata* AgNPs doses for 60 days. Followed by analysis of sperm parameters, fertility, reproductive organ and body weight, testosterone assays and other reproductive health parameters.

Results: The characterization results indicated successful synthesis of AgNPs by their size, shape, crystallinity, elements and stability results. Antifertility activity of AgNPs, showed significant reductions in sperm parameters ($P<0.05$) and fertility, with minimal adverse effects on body weight and haematological parameters. Degenerative changes were observed in histoarchitecture of testis. Testosterone level was affected across various dose levels ($P<0.01$) and markedly reduction in tissue protein, cholesterol and sialic acid was observed. These parameters show a significant recovery in treatment Group-V.

Conclusion: These findings suggest that AgNPs synthesized using *T. undulata* possess promising antifertility potential and can be used as a promising contraceptive agent.

Keywords: Silver nanoparticles, Antifertility, Spermatogenesis, Contraception, *T. undulata* etc

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INTRODUCTION

Worldwide rapid population growth and limited resources have created challenges to sustainable development. An uncontrolled population has generated socioeconomic discrepancies, pollution, and political tensions, posing a threat to humanity's existence. The growing human population highlights the need for advanced control methods [1]. Despite advancements in reproductive medicine, many side effects are associated with present contraceptives methods. Herbal products have been used for centuries as a source of nutrients to promote a healthy lifestyle. There are 4.2 million plant species worldwide, of which 20,000 are considered wild edible and less than 20,000 are used as food supplements by 90% of the population around the globe. In current pharmacopeia, plants or their extracts account for nearly 25% of drug formulations [2]. The World Health Organization (WHO) has also launched a population management initiative, which involves research into traditional methods of medicine. Currently, researchers worldwide are investigating the effectiveness of herbal products as contraceptives because they are less expensive, more effective, and have fewer adverse effects [3].

Nanotechnology is the study and manipulation of materials with dimensions ranging from 1 to 100 nm. The unique properties of this system allow for its novel applications [4]. In the early 21st century, nanoscience and nanotechnology became the emerging fields of research. Nanomaterials have significantly impacted the healthcare system. They are now widely used in drug-delivery systems and pharmaceuticals, including diagnostic, biosensor, and imaging probes at the nano level [5]. Among the many types of nanoparticles, silver nanoparticles (AgNPs) are the most widely employed nanomaterials because of their potent antioxidant, antiviral, antibacterial, antifertility and antifungal activities. Research done by Dziendzikowska *et al.,* 2016, suggested that AgNPs affect the concentrations of Luteinizing Hormone (LH) and sex hormones in the plasma and testes, dramatically reducing dihydrotestosterone and intratestosterone levels [6].

T. undulata, also known as Rohida (Hindi), Desert teak or Marwar teak that belongs to the Bignoniaceae family, a plant of dry and semi-arid region. The plant's methanolic (MeOH) extracts have been shown to be analgesic and anti-inflammatory, as well as effective in treating seizures, cholera, problems related to urinary tract, malaria, heart disorders, and sexually transmitted infections [7]. Compounds of the plants reported are naphthoquinones derivatives, iridoid glucoside, triterpenoids phytosterol, fatty alcohol, flavanols, flavonoid, and glucoside. A previous study by Soni and Mali, 2016, [8], shows oral administration of petroleum ether extract of leaves of this plant, in male albino Wistar rats had antiandrogenic and antifertility effects. Another study conducted by Goyal and Purohit, 2022, [9], found that supplementing male albino rats with root extract of *T. undulata*, exhibits reduced sperm count, density in the cauda epididymis, and motility, leading to decreased male fertility. Therefore, following an in-depth review of the existing literature, current study was methodically designed to explore the antifertility activity of AgNPs synthesized via biogenic process using *T. undulata* MeOH leaf extract. The current research is designed to gain a better understanding of the potential contraceptive applications of these herbal nanoparticles.

MATERIALS AND METHODS

Preparation of plant sample

Collection of *T. undulata* sample (Leaves) was done from in and around the Jaipur district of Rajasthan, India. The plant was identified and validated by herbarium experts at the Department of

Botany, at University of Rajasthan, Jaipur, providing the voucher code RUBL21228. 250 g of the powder was weighed and then used for 50% v/v MeOH extract preparation using methanol (Thermo Fisher Scientific, India, CAS NO. 67-56-1) and milli-Q water in a Soxhlet apparatus (Borosil, India) at 40-45 °C for 24 h (8 h for 3 d) in accordance with procedure suggested by Soni and Mali, 2016 and WHO protocol (1983) [10]. The prepared extract was then filtered through Whatman filter paper and, kept for drying at room temperature (25 °C-30 °C) in an oven, for further use. All chemicals used in the process were of analytical reagent (AR) grade.

AgNPs synthesis

In a beaker 5 ml of a 50% MeOH leaf extract was prepared. Separately, 55 ml of a 1 mmol silver nitrate ($AgNO_3$) (Rankem, India, India, CAS No. 7761-88-8), Solution was made by dissolving 0.017 g of $AgNO_3$ in Milli-Q water. The leaf extract was then added dropwise to the $AgNO_3$ solution under continuous stirring with a magnetic bead. The mixture was heated on a magnetic stirrer at 45±2.5 °C, with constant stirring, for 30 min [11].

Characterization of AgNPs

The synthesized nanoparticles were characterized using a variety of techniques. The characteristic peaks were analysed using UV-visible spectrophotometry, encompassing a range of 300-700 nm with a resolution of 1 nm. The absorbance behaviour of produced AgNPs was evaluated using a Thermofisher Scientific multiskan Go (Version 101.12) spectrophotometer in accordance with protocol followed by Chaudhuri *et al.,* 2016. FTIR spectroscopy was used to explore chemical interactions and identify functional groups; spectrums were created using Perkin Elmer Infra-red spectra 10.4.00, and graphs were merged using Origin Pro version 8. FTIR was performed on KBr pellet mode at 4000-450 cm^{-1} [12]. X-ray diffraction (XRD) analysis was performed to evaluate the crystal structure and determine average particle size. The PANalytical X'pert PRO X-ray diffractometer system was used to study the crystal nature of AgNPs. The material's crystalline lattice was detected using, Cu-Kα radiation at a setting of 40ma/40kv, from a range of 2 theta 20-80̊ [13].

Zeta potential analysis was employed to determine the surface charge and stability of the particles using water as a dispersion, with 12 zeta runs performed in a Zetasizer ver. 7.11 Malvern Instruments Ltd. Further characterization included dynamic light scattering (DLS), DLS/Particle size Analyzer (PSA) is a scientific equipment that measures, illustrates and reports on a synthesized nanoparticle's particle size distribution. The size of the AgNPs were analyzed using size distributor, Malvern (Zetasizer ver. 7.11.) instrument, using water as dispersant, for the duration time of 60 seconds, in a disposable micro cuvette (40 µl), dispersant RI was 1.330 and viscosity of 0.8872 was used [14, 15]. Scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (SEM-EDX), to assess the surface topography, size, shape and composition of elements present, of the synthesize AgNPs, was detected using SEM (Nova nano SEM 450) at magnification of 25000x and HV (High Vacuum) at voltage of 15.0 Kev, the sample was provided as a thin film on a small slide. The elemental purity of nanoparticles was identified using Bruker energy dispersive x-ray (EDX) analyzer [16]. Different elements present in material shows different peaks.

Antifertility activity test

Animals

Forty healthy (4-5 mo old) fertile, colony-bred, Sprauge-Dawely, male, albino Wistar rats (*Rattus norvegicus*), body weight ranging between 150-200 g animals were procured from a certified institute Lala Lajpat Rai University of Vetenary and Animal Sciences (LUVAS), Hisar, Haryana, India. Animals were kept under controlled surrounding for 7 d in quarantine room for their acclimatization, with provision of a 12h/12h light-dark regimen.

Ethical approval

For the welfare, maintenance and use of experimental animals CCSEA and Institutional Animal Ethical Committee (IAEC) guidelines with registration number (1678/GO/Re/S/12CCSEA; Dated: 12/04/2023) for Department of Zoology, University of Rajasthan, Jaipur were followed.

Treatment protocol

Five treatment groups were made for the experiment consisting rats of similar body weight and age. Each group comprise eight animals, and was fed standard laboratory chow and water *ad libitum.*

Dosage preparations

The AgNPs solution was centrifuged, the supernatant removed, and the resulting pellet was sealed in an airtight container and stored at 4 °C for later use. Using this powder, fresh suspension of *T. undulata* AgNPs (10 mg/ml) was prepared daily in distilled water and was delivered through oral route using an oral gavage, following group allocations as mentioned below-

Group-I: Control rats were treated orally for 60 d, with sterile distilled water only.

Group-II: Animals were treated with *T. undulata* AgNPs at a dose of 10 mg/kg B. wt/d for 60 d.

Group-III: Animals were treated with *T. undulata* AgNPs at a dose of 20 mg/kg B. wt/d for 60 d.

Group-IV: Animals were treated with *T. undulata* AgNPs at a dose of 30 mg/kg B. wt/d for 60 d.

Group-V: Animals were treated with *T. undulata* AgNPs at a dose of 20 mg/kg B. wt/d for 60 d and were kept for 30 d recovery period.

Schedule of scarification

Twenty-four hours after their last dose, all surgeries were conducted under mild anaesthesia, with every effort taken to minimize suffering and ensure the well-being of animals.

Experimental parameters

Body and organ weights

All rats were weighed to record their initial and final body weights. The reproductive organs (testis, epididymis, vas deferens, seminal vesicles, and ventral prostrate) were removed from the attached fat and tissue and weighted to the nearest milligram with an electronic machine.

Sperm parameters analysis

To analyse sperm parameters, including motility and density, 50 mg of caudal epididymis was homogenized in 1 ml of Phosphate Buffer Saline (PBS) immediately after of scarification A drop of the uniformly mixed sample was placed on a Neubar Haemocytometer, where sperm motility was assessed by counting motile and immotile spermatozoa per unit area [17]. Following this, epididymal sperm density was analysed using a standard procedure [18].

Toxicological investigation

The counts of RBC, WBC, haemoglobin, haematocrit and standard haematological indices, namely colour index, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) assessed for the normal functioning of vital organs [19].

Tissue biochemical assays

After autopsy, Freezing the tissues allowed for investigation of protein, glycogen, cholesterol, and sialic acid contents. Following Protein [20], Cholesterol [21], Sialic acid [22] methods, were estimated in right side of testis and other accessory reproductive organs, using colorimetric analysis.

Hormone assay

Serum samples was isolated, following blood collection, by standard procedures and stored at-20 °C for subsequent analysis. serum levels of testosterone were further assayed and analysed by using the protocol of the ELISA kits, from Cal biotech [23].

Histopathological studies

For histological analysis, the contralateral side of the testis were fixed in 10 % formalin solution, cleaned, dehydrated in graded

alcohol series, and embedded in paraffin wax. Serial slices of 5 µ were produced from solid block of tissue and stained with Harris Haematoxylin and Eosin stains (H and E) and examined with a light microscope.

Statistical analysis

The statistical analysis was performed using Origin Pro 8 and Microsoft Excel. Data is reported as mean±SEM and statistical significance was determined using Student's t-Test. Findings were classified as significant at (**P<0.01) and (*P<0.05).

RESULTS

AgNPs characterization results

UV visible spectroscopic analysis of a *T. undulata* AgNPs revealed a prominent and strong peak at 434 nm, whereas MeOH leaf extract did not showed any significant peak, in contrast, the $AgNO_3$ solution alone exhibited a baseline in the graph, indicating the successful synthesis of AgNPs, as shown in fig. 1B [24]. The comparative FTIR spectra of both AgNPs and the MeOH extract revealed distinct transmittance patterns, with prominent bands at 3446.62 cm^{-1}, along with several others at 2917–2860.7 cm^{-1}, 1636.28 cm^{-1}, 1384.14 cm^{-1}, and 729.16 cm^{-1}. These bands correspond to various functional groups, including alcoholic O-H, alkane C-H, C=O (aldehyde, ester, or ketone), phenolic O-H, and possibly C=C (alkene) groups, respectively. The comparative spectra (fig. 1D) highlight the presence of key functional groups from plant-derived compounds (secondary metabolites) that act as capping agents, facilitating the successful synthesis of AgNPs [25]. Crystallinity of synthesized AgNPs were detected using, XRD which showed several brags reflection at various 2θ, in the range of 20̊-80̊, at 2 θ = 27.87˚, 32.31˚, 38.17˚, 46.29˚, 57.46˚ and 76.84˚ corresponding to various planes (fig. 1C) as (100), (122), (111), (231), and (311), demonstrates the presence of synthesized AgNPs in the FCC (Face cubic centre) crystalline structure. Sharpening of the peaks indicated their presence in the nano area, when compare with Helfish *et al.*, 2021 [26]. These results show that AgNPs has been synthesized. The crystal size of AgNPs was calculated using full width half maximum, Bragg reflection by Debye-Scherrer equation-D = 0.9λ/βCosθ**,** is 42.07 nm.

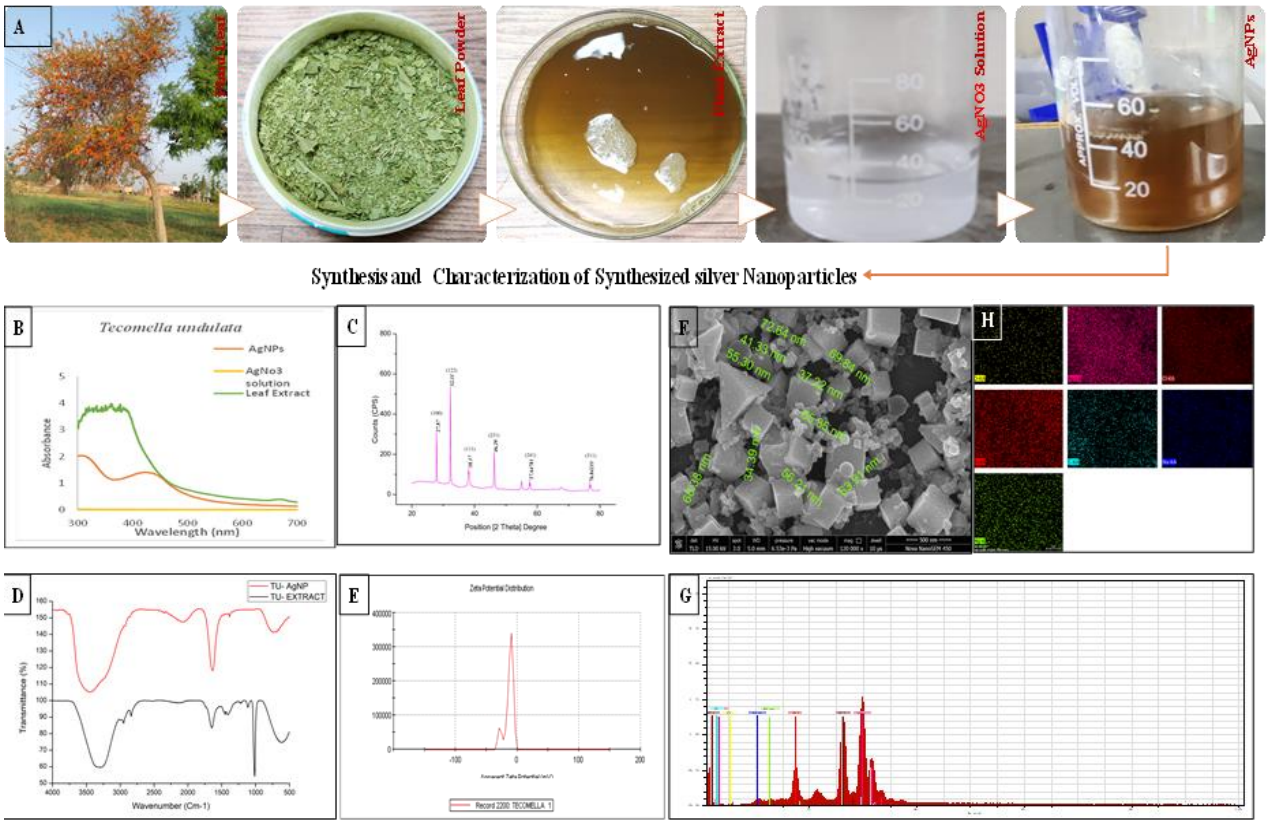

Fig. 1 A: Process of the preparation of the 50 % MeOH Plant extract and synthesis of AgNPs. B: Comparative UV-Visible spectrophotometric analysis for characterization of Synthesized AgNPs with crude MeOH extract and $AgNO_3$ solution. C: X-Ray Diffractogram of AgNPs. D: Comparative FTIR Spectra of *T. undulata* AgNPs and methanolic extract. E: Zeta-potential analysis. F: SEM Imaging G: and H: EDX analysis of *T. undulata* AgNPs and components found on EDX analysis, respectively

Zeta potential analysis showed a single sharp peak at-14.3±7.64 mV (mean±SD) (fig. 1E), at conductivity of 0.212 mS/cm, indicates uniformity of the particles as mentioned earlier by Chaudhuri *et al.*, 2016, higher negative charge confirms the repulsion among the particles, which indicated that AgNPs are stable, and can be stored for long durations. SEM analysis revealed that synthesized AgNPs are spherical in shape, also at some magnification scales it showed aggregations which showed their fractal shapes. The size of particles varied from 34.39 nm-72.64 nm thus, it can be seen nanoparticles are below 100 nm in size (fig. 1F). Further followed by EDX analysis it revealed, the presence of peak at 3 eV reveals the presence of pure AgNPs (fig. 1G), the spectrum results show majorly Ag 59.75%, apart from this other peak of C, O and Mg shows presence of phytoconstituents as capping agent on nanoparticles [27, 28].

Changes in body and organ weights

Oral treatment of AgNPs derived from *T. undulata* MeOH leaf extract in male rats did not cause any significant variations in initial and final body weights of rats. However, as compared to the control Group-I, all treated Groups (Groups II, III, and IV) showed a markedly reduction in the weight of reproductive organs such as the testes, epididymis, vas deferens, ventral prostrate and seminal vesicles in a dose dependent manner. The weight of these organs remains within the control range for recovery Group V (table 1).

Table 1: Changes observed in body and organ weights of male wistar rats treated with AgNPs synthesized from 50% MeOH leaf extract of *T. undulata* for 60 d

Treatment groups	Initial body weight (g)	Final body weight (g)	Testes (mg/100 g)	Epididymides (mg/100 g)	Seminal vesicle (mg/100 g)	Ventral prostrate (mg/100 g)	Vas deferens (mg/100 g)
Group-I (Control)	162.5±3.65	181.25±4.40	1366.88±2.17	547.73±3.89	476.55±4.86	142.13±5.14	146.62±3.27
Group-II (10 mg/kg. B. wt)	160.00±5.34^{ns}	183.75±4.97^{ns}	1345.39±4.68**	521.56±6.71**	455.86±3.53*	123.89±4.23*	126.16±3.52*
Group-III (20 mg/kg. B. wt)	161.25±4.40^{ns}	182.5±5.26^{ns}	1333.78±2.68**	505.92±2.56**	440.53±4.93**	113.10±3.50**	110.65±4.97**
Group-IV (30 mg/kg. B. wt)	163.75±5.32^{ns}	186.25±5.32^{ns}	1307.29±1.54**	489.59±4.40**	423.01±4.91**	101.57±3.81**	95.33±5.22**
Group-V (20 mg/kg. B. wt)	165.00±5.00^{ns}	187.5±5.90^{ns}	1359.56±3.04^{ns}	539.79±5.39^{ns}	464.74±3.02^{ns}	134.77±5.80^{ns}	136.88±4.09^{ns}

(Data are represented as Mean±SEM of 8 animals) Treated Group II, III, IV and V compare with control Group-I. *= Significant at p≤ 0.05, ** =Significant at p ≤0.01, ns=non-significant

Effects on sperm parameters

Cauda epididymal sperm parameters showed evidence of dose dependent effects on sperm motility, as it was significantly diminished in Group II, III and IV. The qualitative sperm motility was reduced and poor quality of spermatozoa movement was observed.

A great decline was also observed in Sperm density, which was significantly higher in Group-IV at a dose level of 30 mg/kg. B. wt. and relatively low for Group-III (20 mg/kg. B. wt.) and Group-II (10 mg/kg. B. wt). After experiment, 30 d recovery period was followed, without administration of any dose, which showed a perfect recovery in Group-V (fig. 2 and 3).

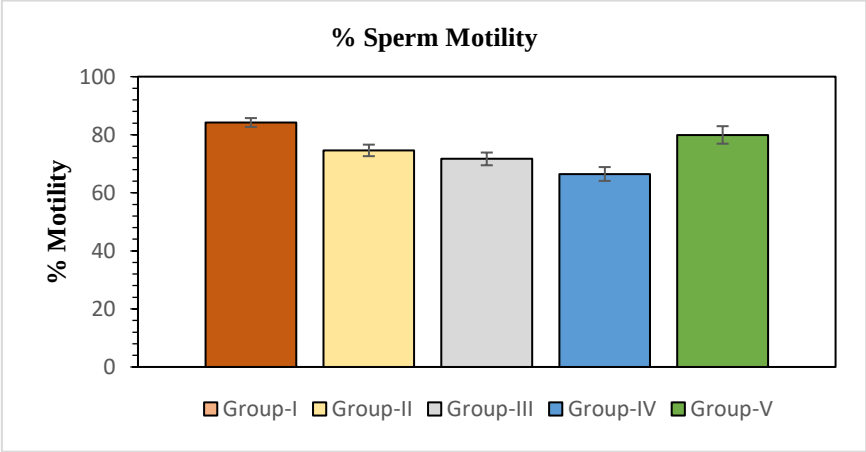

Fig. 2: Changes in % sperm motility, on treatment of *T. undulata* AgNPs,

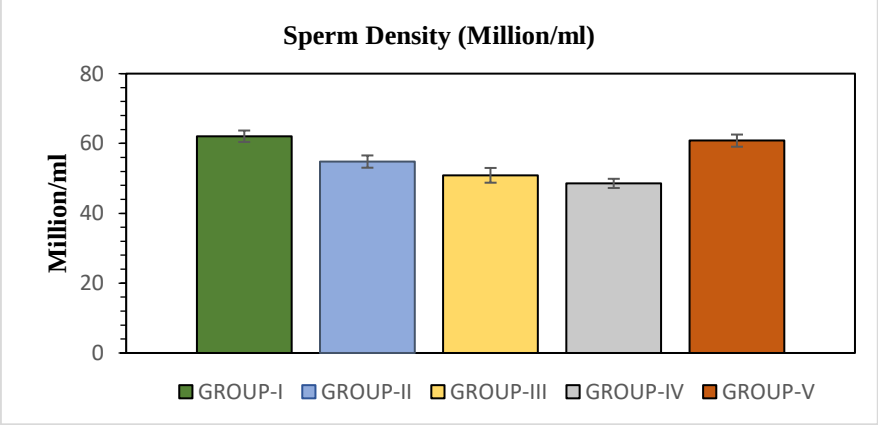

Fig. 3: Changes in sperm density, on treatment of *T. undulata* AgNPs

Toxicological investigations

The haematological parameters across all treatment Groups (Groups II, III, and IV) remain unaltered, when compared with control Group (Group I) indicating that the nanoparticles did not induce any systemic toxicity (Data are not shown here).

Effects on biochemical parameters

Protein concentrations in the testis, epididymis, seminal vesicles, and ventral prostate decreased significantly as the dose increased. When compared to Group I, all treatment Groups had significantly lower concentrations of cholesterol in the Testis and sialic acid in the cauda epididymis. All of these parameters were regained in Group V after a 30 d dose withdrawal period (table 2).

Effects on testosterone levels

The level of serum testosterone decreased significantly in all treatment Groups II, III and IV (P≤ 0.01), compared to vehicle-treated control animals. However, Group-V demonstrated a recovery to normal levels (fig. 4).

Table 2: Changes in tissue biochemical parameters of male rats treated with AgNPs synthesized with 50% MeOH extract of *T. undulata* leaves for 60 d

	Protein (mg/g)				Cholestrol (mg/g)	Sialic acid (mg/g)
Treatment	**Testes**	**Epididymides**	**Seminal vesicles**	**Ventral prostate**	**Testes**	**Epididymides**
Group-I (Control)	277.64±6.27	226.15±3.61	213.71±3.29	188.29±2.55	8.78±0.17	6.52±0.10
Group-II (10 mg/kg. B. wt)	255.16±5.41*	206.10±2.36**	201.39±4.84*	185.95±2.85*	7.82±0.18*	5.76±0.16**
Group-III (20 mg/kg. B. wt)	229.70±2.67**	186.02±3.40**	187.33±4.60**	179.89±2.20**	7.05±0.18**	4.95±0.11**
Group-IV (30 mg/kg. B. wt)	195.20±3.92**	174.85±1.87**	177.82±5.28**	177.69±2.51**	6.34±0.27**	4.05±0.09**
Group-V (20 mg/kg. B. wt)	261.94±5.14ns	218.19±3.46ns	209.09±5.03ns	189.21±1.90ns	8.14±0.26ns	5.88±0.08ns

(Data are represented as mean±SEM of 8 animals) Treated Group-II, III, IV and V compare with control Group-I. *= Significant at p≤ 0.05, ** = Significant at p ≤0.01, ns= non-significant

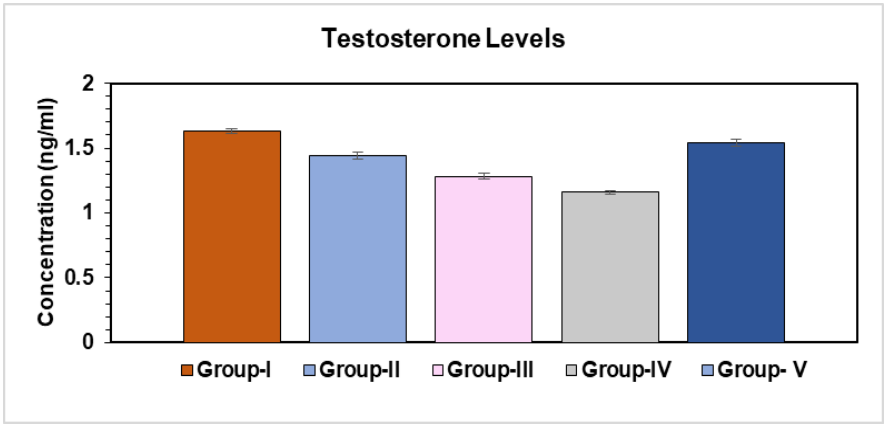

Fig. 4: Change in serum testosterone levels, on treatment of *T. undulata* AgNPs

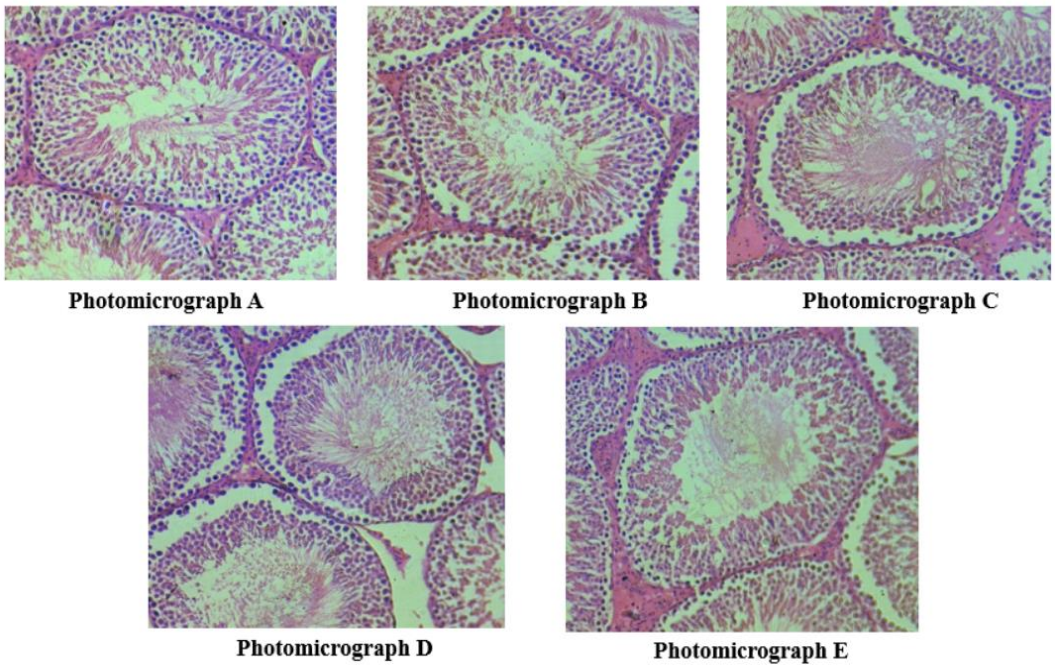

Fig. 5: Histopathological examination of testes at different doses-photomicrograph A (Group-I) shows, A typical histoarchitecture of a control testis with intact Seminiferous Tubules (ST), containing Sertoli cells, spermatogonium, spermatocyte I, spermatocyte II, spermatids and maturing sperms (order-from basal membrane towards lumen), and normal Leydig cells in their interstitial spaces. Photomicrograph B (Group-II) is depicting very minor degenerative changes in testis at a minimum dose. Photomicrograph C (Group-III), decrease in number of Sertoli cells and vacuolization of ST observed, showing detachment from basement membrane, might affecting spermatogenesis process, although diameter of ST did not show any significant variation. Photomicrograph D (Group-IV) Thinning of basal membrane is observed, ST is showing severe vacuolization and distortion, lumen of ST showing decrease in numbers of sperms, oligospermia, degenerative changes are observed also in Leydig cell. Photomicrograph E (Group-V) after dosing for 60 d at 20 mg/kg. B. wt and following no dose period for next 30 d, testis showed a significant recovery, ST are found intact with a thick basal membrane regaining their normal architecture, interstitial tissue is found normal with Leydig cells, lumen have high number of sperms, spermatogonium are located on basal membrane. (H and E, X100)

Histopathological observations

In the control (vehicle-treated) Group-I (Photomicrograph-A), the seminiferous tubules exhibit fully intact germinal epithelium with a well-preserved basement membrane, and the lumen is densely populated with viable spermatozoa. Sertoli cells and embedded spermatogonia maintain their normal morphology and Leydig cells appear fully developed. In Group II (Photomicrograph-B), treated with AgNPs at a dose of 10 mg/kg. B. wt. for 60 d, mild loosening of the germinal epithelium is evident, along with a reduced number of spermatozoa, some of which display degenerative changes. Group III (Photomicrograph-C), shows more pronounced desquamation of the germinal epithelium, while in Group IV (Photomicrograph-D), severe necrosis, extensive structural alterations, a marked reduction or complete absence of functional spermatozoa, and degeneration of Leydig cells was observed. However, after 30 d of treatment withdrawal in Group V (Photomicrograph-E), a significant recovery is observed, characterized by the regeneration of the seminiferous epithelium, restoration of Leydig cell function and an increased number of active spermatozoa, as depicted in photomicrograph E.

DISCUSSION

Results of current study are showing various critical physiological and histopathological effects on reproductive health parameters. A dose-dependent reduction in reproductive organ weights has been observed. An absence of significant changes in body weight and haematological markers indicates that AgNPs were not causing any systemic toxicity, reinforcing their specificity to the reproductive system [29]. Male contraception functions mainly by altering the morphology of sperm to prevent fertilization, rather than stopping spermatogenesis, sperm motility and density, coupled with a decrease in fertility was observed [30].

Several tissue biochemical markers show changes as sialic acid, structural integrity of acrosomal membrane of sperm is dependent on sialic acid, decrease in which indicates change in motility and fertility of sperm. The reduced levels of biochemical parameters in reproductive tissues, such as protein, cholesterol and sialic acid, suggest that the metabolic support for sperm development is impaired. Sertoli cells help regulate the metabolic environment of the seminiferous tubules, and their dysfunction and degeneration could explain the observed reductions in sperm motility and density [31].

Testosterone is a critical hormone (Androgen) for maintaining spermatogenesis and ensuring the proper functioning and development of accessory sex organs, such as the epididymis and ventral prostate, vas deferens, epididymis which contribute to sperm maturation, storage and semen production [32].

Cholesterol is the precursor to all steroid hormones, and any reduction in cholesterol uptake or metabolism can have a direct impact on testosterone production. Reduced serum testosterone levels may also indicate poor steroidogenesis, the biological process by which cholesterol is transformed into steroid hormones, including testosterone, within the testes' Leydig cells. The significant decline in testosterone levels would impair spermatogenesis, contributing to the reduced fertility observed in all the treated Groups. A potential possible mechanism by which AgNPs may exert their antifertility effects could involve by reducing the sensitivity of Leydig cells to the hormonal axis [33-36]. Further this, study found that the antifertility effects induced by AgNPs were reversible. After a 30 d withdrawal period (Group-V), the parameters of sperm density, motility, and testosterone levels showed recovery, suggesting that the effects of AgNPs on the reproductive system were not permanent.

CONCLUSION

In conclusion, these findings provide in depth insight into the potential antifertility effects of AgNPs produced from *T. undulata* MeOH leaf extract in male albino Wistar rats. The delivery of AgNPs resulted in significant dose-dependent changes in reproductive health parameters. Furthermore, the reversibility of these markers in recovery group shows that there may be some restoration of reproductive health parameters after stopping drug delivery of AgNPs. In summary, results of these findings suggest that, this study contribute to the growing research, indicating that AgNPs synthesized from *T. undulata* could potentially be used as a reversible male contraceptive agent.

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AUTHORS CONTRIBUTIONS

All authors have made their substantial and intellectual contribution.

CONFLICT OF INTERESTS

All author declares no conflict of interest.

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