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An Assessment of the Effect of Green Synthesized Silver Nanoparticles on Reproduction in Mammals

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ABSTRACT: This study investigates the reproductive toxicity of rats treated with silver nanoparticles of leaves extract of *P. roxburghii*. Green chemistry was employed for the synthesis of AgNPs using leaf extracts of *P. roxburghii.* The size, shape, morphology, and stability of resultant AgNPs were investigated by UV–Vis Spectroscopy, FTIR, SEM. Suspension of SNPs of *P. roxburghii* leaves were administered orally to albino male rats for 60 days, to determine their effects on the reproductive parameters of the testes. The animals were divided into 5 groups A, B, C, D, and E each group having 10 rats. Group A was control group, and Groups B, C, D were administered 10mg/kg, 20mg/kg, and 30mg/kg b.wt/day respectively of Silver nanoparticles of leaves extract. Group E is recovery group animals of this group treated with medium dose and kept for recovery for 30 days. The UV–Vis spectra showed specific Surface Plasmon Resonance (SPR) absorption peaks between 411 nm and 429 nm which revealed the formation of AgNPs during the synthesis. The nanoparticles synthesis was further confirmed by FTIR and SEM analysis. The results showed that the weight of testes and other reproductive organs were significantly reduced in groups B, C, and D when compared with control. The decrease in the number of sperm cells and sperm motility was greatest in the group D (dose of 30 mg/kg b.wt). Histopathological findings indicated degenerative changes in testes. From these findings, it is concluded that silver nanoparticles of leaves extract of *P. roxburghii* is quite effective in reversible suppression of male fertility.

Keywords: Reproductive toxicity, Silver nanoparticles, *P. roxburghii*, Sperm motility.

INTRODUCTION

Population growth due to unintended pregnancies and it's deleterious socioeconomic and health effects is major concern in the entire world (Kansotiya *et al*., 2023). The 2011 census of India was the world's second-largest ever, trailing only China's census the year before. In recent decades, India's growth rate has slowed due to factors such as growing urbanisation, rising levels of education, especially among women, and greater efforts to alleviate poverty (Abbas *et al*., 2019; Soni and Mali 2017). The population of India is expected to surpass that of China by 2026, when both nations would have a combined total of around 1.46 billion people, notwithstanding recent slowdowns in the country's population growth. Family planning has been supported by a number of contraceptive techniques, including copper-T, condoms, diaphragms, tubectomy, and contraceptive tablets (Opeyemi *et al*., 2024). These methods are mostly female oriented. Contraceptive pills are usually female sex hormone like estrogen, progesterone or their derivatives single or together have been available for a long time, but there is still a search for new ones (Sharma *et al*., 2015). Current methods of contraceptive result in an unacceptable rate of unintended pregnancies. Approximately 50% of all pregnancies are unintended at conception; 50% of those occur in the 94% of sexually active couples who report using some method of contraception (Uwejigho *et al*., 2023).

Numerous plants were previously utilised to reduce fertility, and current scientific research has confirmed that at least some of the researched herbs have these effects. The development of safe, highly effective, and affordable contraceptive agents has been the focus of research, as one of the many alternative family planning strategies. The fascinating field of nanotechnology is revolutionizing social progress to the maximum extent achievable by encouraging new inventions in every element of life. Nanotechnology has the ability to replace conventional methods in a cost-effective and high-performing manner with a highly efficient, eco-friendly, sustainable, and sustainable process (Wang *et al*., 2021). Due to their unique optical, thermal, mechanical, structural, and electromagnetic properties, nanomaterials offer a wide variety of potential applications. Throughout the course of therapy, nano-systems have the ability to deliver the active ingredient to the targeted site of action at a concentration that is sufficiently.

Some studies reported that the gold nanoparticles penetrates the head and tail of the sperm and produces a diminution in sperm motility (Wiwanitkit *et al*., 2009). Aluminum, silver, and molybdenum trioxide were toxic to mouse spermatogonial stem cells (Ema *et al*., 2010). Exposure to nanoparticles also affects the male reproductive system, including an impact on spermatogenesis from the point where it begins in the seminiferous tubules of the testicles. In addition, exposure to NP reduces sperm formation (Boisen *et al*., 2013). According to Kong *et al*. (2014), the rats were given oral gavages containing nickel nanoparticles, and the exposure group's appearance of reproductive cellular apoptosis, sperm motility, and sex hormone levels were all monitored. It is also known that silver nanoparticles have extremely harmful effects on the male reproductive system. According to McAuliffe and Perry (2007), they have the ability to enter the bloodstream and settle in the testes, where they have an adverse effect on sperm cells. Research focusing on AgNPs has been linked to detrimental effects on male reproductive in models of mammals (Lafuente *et al*., 2016). Rats exposed sub-chronically orally to AgNPs coated with polyvinyl propylene also exhibit defects in sperm morphology and changes in testicular histology (Hong *et al*., 2016).

According to Marimuthu *et al*. (2020); Liao *et al*. (2019), silver nanoparticles (AgNPs) have numerous important biological roles in the fields of therapeutics (antimicrobial, anticancer, anti-parasitic, antidiabetic, and antioxidant activities), bimolecular detection and diagnostics, drug delivery, food production, agriculture, and waste treatment. Because of their physical, chemical, and biological characteristics, silver nanoparticles (Ag-NPs) are widely used in a variety of applications, including food packaging, anticancer medications, antibacterial agents, dye effluent treatment, and industrial applications (Choudhury *et al*., 2020). Synthesis of nanoparticles from plants has been gaining importance in recent years due to their solvent free nature and less toxicity (Tesfahuneygn and Gebremichael 2019). Recently, metal nanoparticles were applied in shampoos, soaps, detergents, cosmetics, toothpaste and medical and pharmaceutical products (Tortella *et al.*, 2020).

Putranjiva roxburghii traditionally been used to treat mouth and stomach ulcers, hot swellings, and small pox, as well as for burning sensations, ophthalmopathy, hyper emesis, elephantiasis, impairment, strangury, azoospermia, ordinary termination, and infertility. Orally, women eat *Putranjiva* nuts to influence the delivery of a male child. Several investigations in the fields of biological industries and pharmaceutical applications have been conducted on the production of silver nanoparticles utilising medicinal plants (Falchi *et al*., 2018; Thakur *et al*., 2014). In present study Ag nanoparticles synthesised by biological method using plant *Putranjiva roxburghii* belongs to euphorbiaceae family and determined their antifertility activity in male rat.

MATERIAL AND METHODS

Animal Model. Colony-bred, 3-4 months old Wistar strain albino rats (*Rattus norvegicus*) weighing between150-200gm were procured from Bilwal Medchem Research Laboratory Pvt Ltd, Sikar, Rajasthan, India. The animals were quarantined for 14 days by housed in polypropylene cages, measuring 430×270×150 mm, under controlled environment situation. The animals were kept under hygienic condition and maintained at a temperature of 20 ± 5˚C relative humidity of 50±5% and 12 hrs. dark-light cycle.

Collection & Identification of plants material. The fresh leaves of *P. roxburghii* were collected in enough, from University Nursery, UOR, Jaipur, Rajasthan, India. For the identification and authentication the specimen voucher of *Putranjiva roxburghii* were deposited at Herbarium of Department of Botany, University of Rajasthan, Jaipur. The plant identified and authenticated as –*Putranjiva roxburghii* **(RUBL211757)**.

Synthesis of silver nanoparticles. For the synthesis of silver nanoparticles, fresh plant leaves washed in running water and shade dried then crushed mechanically to prepare 50% methanolic extracts according to the WHO protocol CG-04 (WHO, 1983). The leaves extract of plant and silver nitrate solution was used (Suman *et al*., 2013). The primary detection of synthesize silver nanoparticles carry out in the reaction mixture by using observing the color change of the medium from greenish to dark brown. After each hour, a small amount of reaction mixture was centrifuged, the supernatant was collected and pellet store. Collect supernatant heat and the change in color, indicating the formation of nanoparticles.

Characterization of silver nanoparticles. In this study, biophysical spectroscopic techniques were utilized to analyze the properties of the samples.

UV analysis. Synthesized silver nanoparticles were scanned by UV-Vis spectrophotometer at the wavelength of 300-700 nm. UV-Vis spectroscopy used for metal particle surface plasmon resonance absorption in visible region**.**

FT-IR analysis. FT-IR spectra were recorded at room temperature using a Bruker Alpha FT-IR instrument (Bruker Corporation, Massachusetts, USA), covering a spectral range of 4000–500 cm^{-1}. The FTIR sample were prepared in liquid form as synthesized.

FE-SEM analysis. Field emission scanning electron microscopy (FESEM) images were captured with a JSM-7610F Plus electron microscope, operated at an accelerating voltage of 5 kV.A thin film of the sample were prepared by just dropping small drop on grid then the sample was allowed to dry and image was taken.

Treatment Protocol. In this experiment 50 animals were divided equally into five treatment groups, each consisting of 10 animals.

Group-A: The animals were given vehicle (sterile distilled water) alone orally for 60 days to serves as vehicle controls.

Group-B: The animals were treated with silver nanoparticles of *P. roxburghii* leaves extract at a dose of 10 mg/kg.b.wt./day for 60 days.

Group-C: The animals were treated with silver nanoparticles of *P. roxburghii* leaves extract at a dose of 20 mg/kg.b.wt./day for 60 days.

Group-D: The animals of this group were treated with silver nanoparticles of *P. roxburghii* leaves extract at a dose of 30 mg/kg.b.wt./day for 60 days.

Group-E: The animals were administrated silver nanoparticles of *P. roxburghii* leaves extract a dose of 20 mg/kg.b.wt./day for 60 days will be kept for a recovery period of 30 days.

STUDY PARAMETERS

Fertility Test. Successful mating was carried out with all the animals 5 days prior to sacrifice (male female ratio 1:2). The number of pups were recorded and litter size and percent fertility was calculated (WHO, 1983).

Sperm Motility and Density. For sperm motility and density, 50 mg of cauda epididymis was minced in 1 ml of physiological saline. The percent motility was determined by counting both motile and immotile spermatozoa per unit area. After this, cauda epididymal sperm density was made by routine procedure and express as millions/mm^3 suspension (WHO, 1983).

Reproductive Organs Weight. Testes, epididymis, seminal vesicles and ventral prostrate were dissected out, freed from adherent tissues and weighed to the nearest milligram on an electronic balance.

Tissue Biochemistry. The testis, epididymis, seminal vesicles, vas deferens and ventral prostrate were freezed for the analysis protein (Lowry *et al*., 1951) and sialic acid (Warren, 1959) contents.

Histopathology. Contra-lateral side of the testis was fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Sections are cut at and stained with Harris's hematoxylin and eosin and observed under a light microscope.

Statistical analysis. Data were demonstrating as mean ± S.E. and student's "t" test was used for statistical significance. The data considered as significant and highly significant at $p \leq 0.01$ and $p \leq 0.001$, respectively (Gupta, 1978).

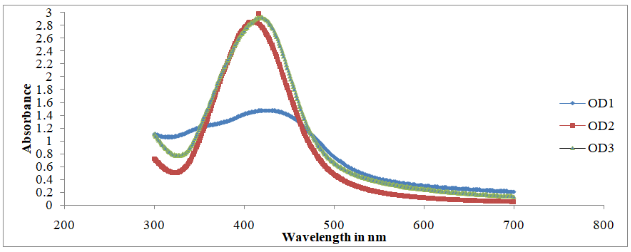
Ethical aspects. The study was carried out with project proposal No. UDZ/2021/14 under the supervision of Institutional Animal ethical committee (IAEC) of the Department of Zoology, University of Rajasthan, Jaipur and CPCSEA guideline was followed (CPCSEA, 2006).

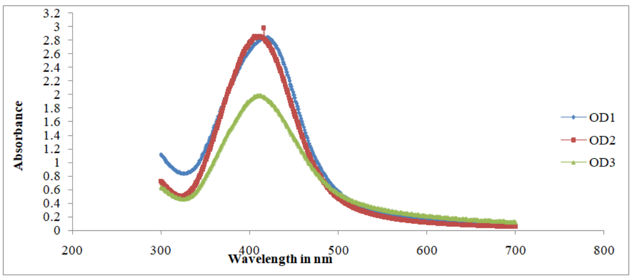
RESULTS

Synthesis of Silver Nanoparticles. 50 ml of Silver nitrate solution is prepared for standardization and synthesis of silver nanoparticles. $AgNO_3$ Solution of 1Mm prepared by adding silver nitrate (RANKEM, PO91G04) in de-ionized water in a fix ratio. Extract Solution prepared by adding Extract in 50% methanol in a fix ratio. There were two groups performed, each in triplicate manner. The Silver Nanoparticles synthesized against temperature and conc. parameters. In each group a beaker filled with water and put test tubes marked A, B, C. In each test tube added $AgNO_3$ Solution of 1Mm after that add extract solution in fix ratio and heated on 65-70°C on hot plate. The colour of solution was changed in dark brown, this indicated SNPs were synthesized. As a consequence, SNPs of *P. roxburghii* leaf extract were fabricated.

Silver Nanoparticle Characterization. Synthesized silver nanoparticles exhibited incredibly impressive dimensions, ranging from 10.7 nm to 65.2 nm in size, and they were characterised using analytical techniques such UV-Vis spectrophotometer, FTIR spectra and SEM spectroscopy from Manipal University Jaipur, Rajasthan 303007, India.

UV-Vis spectroscopy Results. During our analysis, we observed particular Surface Plasmon Resonance (SPR) absorption peaks in the UV-Vis spectra between 411 and 429 nm, which indicated the production of Silver NPs. A prominent, broad peak was seen in the UV–visible spectra at 423 nm; the peak's broadening suggested that the particles were poly-dispersed. Because of surface plasmon resonance, AgNPs are known to display UV–visible absorption maxima in the 400–500 nm range.





FE-SEM Results. Using a variety of magnification settings, SEM was used to assess the silver nanoparticles' size and shape. Ag-NPs create irregularly shaped, agglomerated nanoparticles, as shown in the photos, and this is verified by SEM examination.

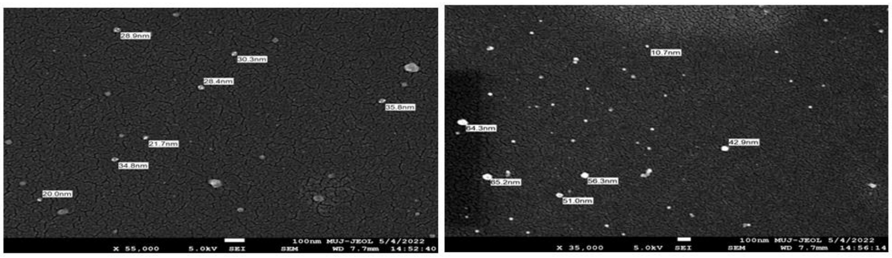


Fig. 1(A & B). SEM analysis of SNPs of leaves extract of *P. roxburghii* ranging from 10.7 nm to 65.2 nm in size.

FTIR Results. The presence of biomolecules that may possibly aid in the reduction of Ag^+ ions or the capping of the bio-reduced AgNPs was determined using FTIR spectra. The two bands observed at 1,380 cm^{-1} and 1,030 cm^{-1} can be assigned to the C–N stretching vibrations of the aromatic and aliphatic amines, respectively. FTIR spectra of AgNPs synthesized from plant extract, with peaks at 3324 cm^{-1} is typically attributed to the stretching vibration of a free O-H bond in alcohol. Amide or peptide bands are major bands of the protein infrared spectrum. The amide band (between 1600 and 1700 cm^{-1}) is mainly associated with the C=O stretching vibration (70-85%) and is directly related to the backbone conformation. AgNPs of different sizes showed similar spectra without any significant differences.

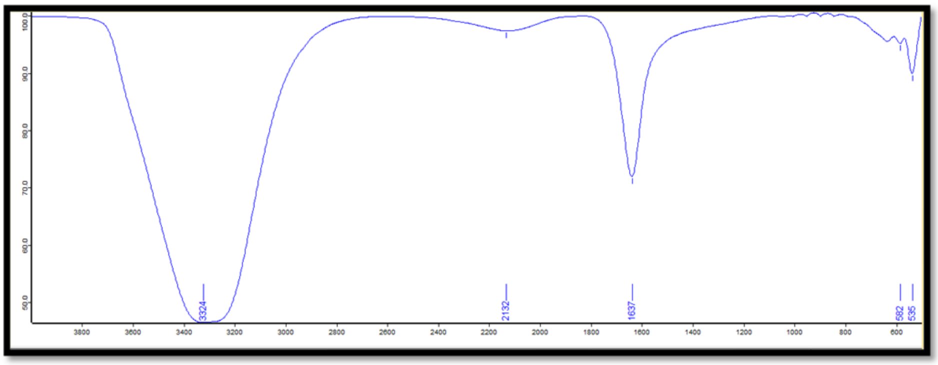


Fig. 2. FTIR spectra of SNPs of leaves extract of *P. roxburghii.*

Antifertility effect of SNPs of *P.roxburghii.* In this investigation, we examined body and their reproductive organs weight and the motility and density of their sperm cells. The outcomes showed that Ag-NPs had anti-fertility effects and inhibited male rat spermatogenetic behaviour.

SNPs effect on reproductive organ weights. The weight of the testes and other secondary reproductive organs reduced with the therapy of AgNPs but body weights did not change much.

Table 1: Change Organs Weight of male albino rats after treated with SNPs of leaves of *P. roxburghii.*

Treatment	Testes	Epididydemes	Seminal Vesicle	Ventral Prostate	Vas Deferens	Body Weight[GM]	
	Mg/100gm					Initial	Final
Group-A Control Vehicle Treated	879.62±7.59	595.15 ± 5.66	481.21 ± 9.34	174.88 ± 4.27	171.41 ± 4.14	134 ± 4.13	142.5 ± 3.78
Group-B 10mg/kg b.wt/day	830.65±8.65*	509.43 ± 5.33*	436.86 ± 9.70*	156.47 ± 6.00 ns	143.26 ± 4.67 ns	157.9 ± 9.70	173.5 ± 9.81 ns
Group-C 20mg/kg b.wt/day	813.39±6.15*	502 ± 8.96**	400.57 ± 2.08***	141.78 ± 4.66 ns	130.02 ± 1.45 ns	153.8 ± 9.70	172.9 ± 10.25 ns
Group-D 30mg/kg b.wt/day	688.99±9.76* **	488.54 ± 8.12**	373.36 ± 8.94***	134.48 ± 4.78**	124.24 ± 6.56*	151 ± 6.82	164.9 ± 8.24 ns
Group-E 20mg/kg b.wt/day (30 Days)	863.70± 7.30 ns	574.30 ± 7.57 ns	470.30 ± 4.87 ns	168.62 ± 4.93 ns	161.08 ± 4.06 ns	143.2 ± 2.58	156.3 ± 2.44 ns

(Mean±SEM of 10 animals) ; Group B, C, D, E Compared with Group-A
***= Highly significant (p≤0.001); **= Significant (p≤0.01); *= Significant (p≤0.05); Ns= Non significant

SNPs effect on sperm dynamics & Fertility. When male rats were given *P. roxburghii* leaf SNPs for 60 days, there was a substantial decrease in both cauda epididymal sperm concentration ($p \leq 0.001$) and motility ($p \leq 0.001$) at 30 mg/kg.b.wt./day treatment groups. Following treatment with SNPs derived from *P. roxburghii* leaf extract, the reproductive rate of treated rats decreases by up to 80%. The declined number of fertile males further support to reduce sperm concentration and motility.

Table 2: Change in Sperm Dynamics and Fertility test of male albino rats after treated with SNPs of leaves of *P.roxburghii*

Treatment	No. of Mated Males	No. of Mated Females	No. of Pregnant Females	Sperm Motility (Cauda) [%]	Sperm Density (Cauda) [mil/ml]	Fertility Test[%]
Group-A Control Vehicle Treated	10	20	19	68.54 ± 0.82	46.76 ± 0.98	95%
Group-B 10mg/kg b.wt/day	10	20	16	63.48 ± 0.56 ^{ns}	42.46 ± 0.42 ^{ns}	80%
Group-C 20mg/kg b.wt/day	10	20	10	62.72 ± 2.82**	39.42 ± 1.79**	50%
Group-D 30mg/kg b.wt/day	10	20	4	59.34 ± 0.66***	33.57 ± 0.61***	20%
Group-E 20mg/kg b.wt/day (30 Days)	10	20	18	66.03 ± 1.01 ^{ns}	46.36 ± 1.32 ^{ns}	90%

(Mean±SEM of 10 animals) ; Group B, C, D, E Compared with Group-A
***= Highly significant ($p \leq 0.001$)
**= Significant ($p \leq 0.01$); *= Significant ($p \leq 0.05$); Ns= Non significant

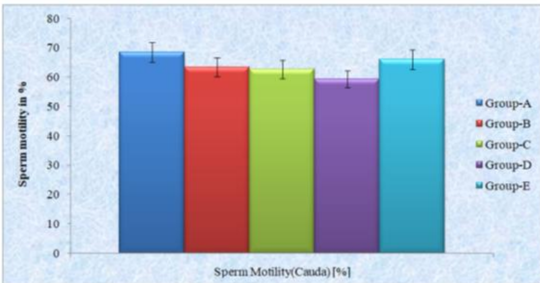


Fig. 3. Comparative analysis of sperm motility in various treatment groups.

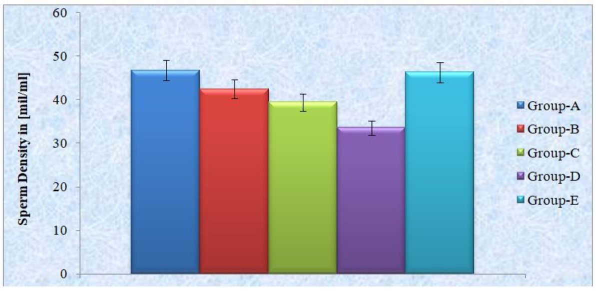


Fig. 4. Comparative analysis of sperm density in various treatment groups.

SNPs effect on protein and sialic acid content. The current study found that the administration of silver nanoparticles containing *P. roxburghii* leaf dramatically reduced the protein content in the testes and other sex organs This finding raises the possibility that the testes lack some stages of spermatogenesis (Soni and Mali 2017).

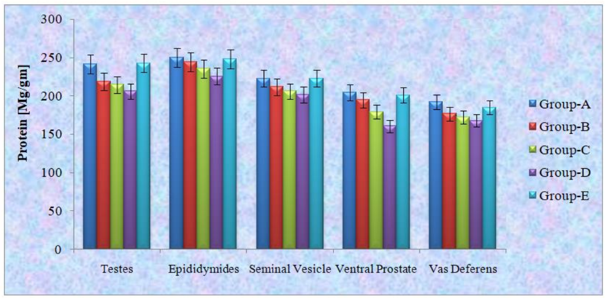


Fig. 5. Comparative analysis of protein content in various treatment groups.

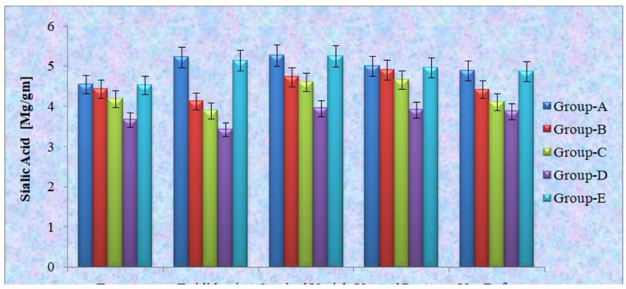
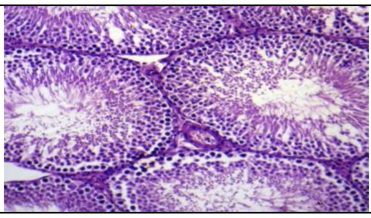
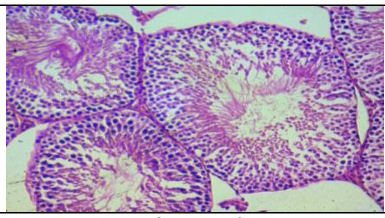


Fig. 6. Comparative analysis of Sialic acid content in various treatment groups.

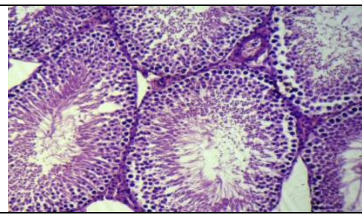
AgNPs effect on histopathology of the testes. Silver nanoparticles synthesized from leaves of *P.roxburghii* have harmful effect on histology of testes of rats.



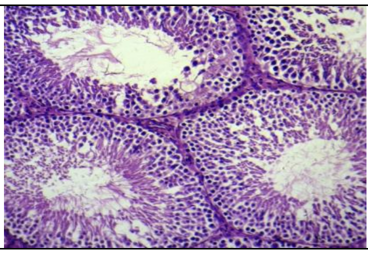
Histopathology of the testis of a control animal. The seminiferous tubules contain Sertoli cells and germ cells of various stages, covering the entire process of spermatogenesis.



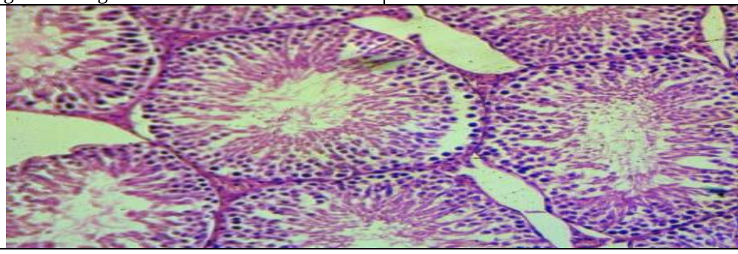
Histopathology of testis of rat treated with treated with silver nanoparticles of *P. roxburghii* leaves extract10 mg / kg.b.wt/rat. Shows structural disorganization and degenerative germinal of testis



Histopathology of testis of rat treated with silver nanoparticles of *P. roxburghii* leaves extract 20 mg / kg.b.wt/rat.



Histopathology of testis of rat treated with SNPs of *P. roxburghii* leaves 30 mg / kg.b.wt/rat. Shows Intertubular space was enlarged in between seminiferous tubules and reduced sperm number in lumen.



Histopathological architecture of testis of rat kept for recovery shows normal seminiferous tubules, sertoli cells.

As per the results in our study and other studies, AgNPS may have caused degenerative histopathological change by production of free radicals and oxidative stress (Alyassery and Hasan 2021.)

DISCUSSION

In our examination, significant reductions in the weights of the reproductive tissues were indicative of an interrupted microenvironment of the testis and epididymis tissue as a result of extract administration, as indicated by decreases in sperm density and motility (Soni and Mali 2017). According to several studies as Mozafari *et al*. (2020); Lafuente *et al*. (2016); Falchi *et al*. (2018), Ag-NPs have a negative impact on reproductive indicators, including an increase in abnormal sperm, changes to the structure of the testes, a decrease in spermatogenesis gene expression, a decrease in serum testosterone, an increase in germ cell apoptosis, and a disruption of the hormonal regulation of reproductive processes in male rats.

According to results, a significant decrease was observed in the litter number when SNPs of leaves extract of *P. roxburghii* were administered. This decrease may have been due to its reported abortifacient effect, causing the abortion of foetuses (Ankul *et al*., 2020; Ahmed *et al*., 2017; Elsharkawy *et al*., 2019). The role of SNPs of leaves extract of *P. roxburghii* on male fertility at various dose considered in our research is similar to result obtained from a previous study where the extract significantly reduced sperm count and motility of male animals. At this dosage, mild to moderate cellular changes in the testes have also been reported on treated rats when ascertained on histological examinations disclosing its toxic attributes of cellular damage to organs and diminution in male reproductive capability (Omotoso *et al*., 2017).

Reduces in androgen may be linked to low levels of sialic acid in the testes, epididymides, and seminal vesicles in treated rats. Reduced sialic acid concentration may result in modifications to the structure of the acrosomal membrane, which may have subtle impacts on spermatozoa motility and metabolism (Uwejigho *et al*., 2023). Based on the findings of our investigation and previous research, SNPs in leaf extract may have changed the histology by generating free radicals and oxidative stress. It has been discovered that AgNPs cause the generation of ROS, which prevents membrane oxidation, DNA damage, protein carbonylation, and regenerated glutathione (GSH) (Ahmed *et al*., 2017).

CONCLUSIONS

From the results of current investigation, it can be concluded that SNPs of leaves extract of *P. roxburghii* is effective in the terms of male contraceptives due to their fertility regulative. The effects may have an inhibitory influence on surrounding environment of testis which may be responsible for the decline in sperm density and motility, leading to changes in spermatogenesis.

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