RESEARCH PAPER

Zinc oxide nanoparticle assisted Mucuna pruriens therapy for male fertility regulation

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ABSTRACT

Background: *Mucuna pruriens* seeds are known to contain diverse Phytoconstituents with antioxidant and reproductive health supporting properties. Male infertility, frequently arising from oxidative imbalance, endocrine disruption, and defective spermatogenesis, remains a growing clinical concern. The present investigation examined the fertility enhancing efficacy of zinc oxide nanoparticle (ZnO-NPs) coated with *Mucuna pruriens* seed extract synthesized through a green approach.

Methods: A 70% ethanolic extract of the seeds was subjected to phytochemical screening and utilized for nanoparticle fabrication. Biosynthesized ZnO nanoparticles exhibited a mean particle size of 149.19 nm with a polydispersity index of 0.112, indicating homogeneous distribution. The recorded zeta potential of −8.7 mV suggested surface functionalization by phytochemicals and moderate colloidal stability. SEM observations demonstrated irregular quasi-spherical particles with slight aggregation, confirming successful nanoparticle formation. Male reproductive impairment was induced in Wistar rats using Ornidazole, followed by oral administration of the extract and its nano-formulation for 24 days in decided groups of rats. Parameter related to fertility, including testis weights, sperm count, motility, viability, morphology, serum reproductive hormones, and testicular histology were evaluated.

Results: The ZnO-NP-coated extract markedly improved sperm quality, and restored testicular structure when compared with intoxicated controls. Results show that the Nano formulation exhibited greater efficacy than the crude extract, suggesting improved bioavailability.

Conclusion: These findings support the therapeutic potential of *Mucuna pruriens*-based zinc oxide nanoparticles in managing male reproductive dysfunction.

Keywords: Mucuna pruriens, Zinc oxide nanoparticles, Green synthesis, Male infertility, Spermatogenesis, Phytochemicals, Nano-phytotherapy.

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1. Background:

A couple is usually diagnosed with infertility if they have unprotected sex for at least a year without becoming pregnant. Men account for half of the 9% of infertile couples, according to WHO estimates that 72.4 million individuals are affected (Boivin et al., 2007). The reasons of male infertility can be classified into two groups: normal spermograms (10–20%) and aberrant spermograms (65–80%), of which the aetiology may not be determined. According to Winters and Walsh (2014), sperm and hormonal transfer may account for an additional 10%. Obesity is another lifestyle factor that contributes to male infertility because it disrupts the estrogen to testosterone ratio (Bieniek et al., 2016). Smoking, drug abuse, and the use of illegal substances also play a role, albeit a causal association has not yet been established (Yu et al., 2018). Other aspects of lifestyle, such as diet, coffee use, and degree of activity, can affect infertility. Sperm quality has been demonstrated to improve with lifestyle modifications, such as reducing red meat consumption, increasing vegetable consumption, and quitting smoking, junk food, and excessive alcohol usage (Gameiro et al., 2016; Cairo Consensus Workshop Group, 2020). Environmental factors, such as cell phones and pesticides, have also been linked to infertility (Gameiro et al., 2016; Bieniek et al., 2016). As a result of these factors, infertility has become a worldwide concern, and many herbal medicines from the Unani and Ayurvedic systems are used to treat it. Numerous bioactive substances have been found in Mucuna spp. The plains and forests of India are home to over fifteen different species of Mucuna (Raina and Khatri 2011). Numerous countries employ Mucuna pruriens (L.), a member of the Fabaceae family, in traditional medicine (Y. Rajeshwar et al., 2005;

GJ. Longhi et al., 2011). According to Mishra and Wagner (2004), the plant is a herbaceous twining annual that grows in hedges and bushes in damp places, ravines, and rubbish jungles over the plains of India. The seeds of the Mucuna plant have drawn particular attention, despite the fact that other parts of the plant have been thought to have medicinal properties. Along with a high protein content (26–29%) and other nutrients, seed also includes substantial levels of bioactive compounds such free phenolics, tannins, and phytic acid (Pugalenthi et al., 2007). Ethnic communities in Tamilnadu, Kerala, Karnataka, and Andhra Pradesh have long eaten the mature seed (Vadivel and Biesalski, 2012). Seeds are used in traditional medicine as a tonic and to boost male energy. *M. pruriens* is also employed in the Unani and Ayurvedic systems of Indian medicine to treat male impotency, sexual debility, and as a nervine tonic (Mishra and Wagner, 2007; Thakur et al., 1989).

The leaves and pods are used by certain tribal groups in Nigeria as food. (Adebowale K.O. and Lawal O.S, 2003). It is utilized as an aphrodisiac and nervine tonic in India (Mishra L, Wagner H., 2007). Mucuna Pruriens seeds are also used by a number of food firms to thicken soups (Ukachukwu et al., 2006). According to earlier research, Mucuna pruriens seeds may destroy hepatocytes in a dose-dependent manner (Cronwall R et al., 1980). The plant's roots are used as a febrifuge and tonic for nephropathy, strangury, dysmenorrhea, amenorrhea, elephantiasis, dropsy, neuropathy, ulcers, and fever. This plant's leaves are used to treat ulcers, inflammation, helminthiasis, cephalalgia, and general debility. They are also thought to be tonic and aphrodisiac. Snakebite, sexual debility, cough, TB, impotence, rheumatic disorders, muscular discomfort, gonorrhea, sterility, gout, psychosis, dysmenorrhea, diabetes, and cancer are all treated using the seeds of this plant (Rastogi RP and Mehrotra BN 1994; Warrier PK et al., 1995). This plant is known to be useful and taken to cure many health problems from ancient time.

2. Methods:

2.1 Plant Sample Collection and Identification:

2.1 Plant Sample Collection and Identification: Triphala Store provided *Mucuna pruriens* seeds, which were purchased at a nearby herbal market in Jaipur, Rajasthan, India. Through morphological comparison with reference specimens from the University of Rajasthan's Department of Botany, the plant material's botanical identity was confirmed. A voucher specimen **(Voucher No. RUBL21701)** was placed in the departmental herbarium for future reference and documentation after the authentication was verified by a trained taxonomist.

2.2 Processing of plant materials: Distilled water and standard analytical-grade ethanol were used for extraction. Water has food value and ethanol has well-known therapeutic properties, which is why the two solvents were selected. Additionally, the bioactive components of *M. pruriens* have been found to be commonly extracted by ethanol and water (Lampariello, L.R et al., 2011). 500 mL of 70% hydroethanol and about 25 g of crushed *M. pruriens* seeds were added to conical flasks. For 48 hours, the conical flasks and their contents were agitated in an orbital incubator shaker. Whatman No. 1 filter paper was then used to filter the crude extracts in a Buchner funnel that was connected to an electric vacuum pump. After being cooled to -40°C, the aqueous filtrate was freeze-dried for a full day. A rotary evaporator was set to 78 °C to dry the ethanolic filtrate. Before being used, the extracts were kept in sterile bottles at 4°C in the refrigerator (Jimoh, M. A. et al., 2020).

2.3 Extracts quality assessment: Carbohydrates, saponins, phenolic substances, tannins, flavonoids, proteins, amino acids, gums, and mucilage were all identified qualitatively in the *M. pruriens* seed extract. In order to standardize each batch of produced extract, the pH was adjusted to around 10 by adding sodium hydroxide solution dropwise to a 100 mL flask containing about 20 mL of the seed extract solution. Another 0.05 M [Zn(CH3CO2)2] solution in 20 mL. To ensure a homogenous solution, heated to 80 °C while being steadily stirred on a magnetic stirrer. After that, a 20 mL solution of *M. pruriens* extract was added drop wise to the [Zn(CH3CO2)2]•H2O, and the mixture was heated to 85°C for two hours. The reaction came to an end when the solution's color changed from black to brown throughout the heating process. After cooling to ambient temperature and centrifuging the mixture for ten minutes at 10,000 rpm, the product was separated by three rounds of washing with an ethanol and water solution. The product was dried in the oven at 50 ℃ for 3 h, and thereafter calcinated at 350 ℃ for 2 h to afford ZnO NPs. All the steps are shown in Figure 1. It involves steps for Biosynthesis of ZnO nanoparticle using *Mucana prureins* seeds extract.

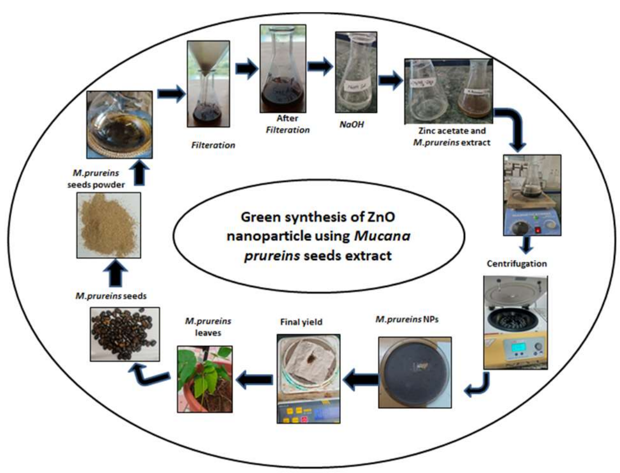

Fig. 1 Schematic illustration of steps for Biosynthesis of ZnO nanoparticle using *Mucana prureins* seeds extract

2.4 Experimental Animals:

Both sexes of healthy Swiss albino rats (Wistar Strain) were used in the study; males were used for the main experimental evaluation and females were assigned to the oral acute toxicity assessment. The animals weighed between 250 and 300 g at the time of the trial, and they were between 8 and 12 weeks old. Rats were kept in conventional polypropylene cages that could hold six animals each. They were kept in controlled environments with a temperature of 25 ± 3 °C and a natural 12-hour light-dark cycle. During the experiment, drinkable water and a standard laboratory pellet meal were available at all times. All animals were acclimated to the laboratory conditions for ten days before to the start of the investigation. The animals were given free access to water during an 18-hour fast prior to experimental procedures, in compliance with the Organization for Economic Co-operation and Development's (OECD) recommendations (OECD 1996). Diethyl ether was carefully administered to produce anesthesia in order to collect blood samples. According to normal anesthetic protocols, the animals were exposed to about 3.20% minimum alveolar concentration (MAC•[1]) of diethyl ether in a glass-covered induction chamber to induce anesthesia (Flecknell, 2015; Miranda et al., 2011). The National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH, 2010) contains ethical standards and principles that were strictly adhered to during all experimental procedures. Before any in vivo research began, the Institutional Animal Ethics Committee (IAEC) of NIMS University Rajasthan, Jaipur, granted ethical authorization for the study under approval number **[NIMSUR/IAEC-01/2024/05].**

2.5 Grouping and dosing of experimental animals:

Table 1 Grouping and dosing of experimental animal, OZ – Ornidazole, CC – Clomiphene citrate

Sr. No	Groups	Rat count (Swiss albino rat of Wistar strain)	Dose
1.	Control	6	10 ml/ kg Dist water
2.	Infertile (OZ)	6	10 ml/ kg Dist water (200mg/kg)
3.	Standard (CC)	6	10 ml/ kg Dist water (50mg/kg)
4.	*Mucana prureins* seeds extract (Low dose)	6	10 ml/ kg Dist water (250 mg/kg)
5.	*Mucana prureins* seeds extract (High dose)	6	10 ml/ kg Dist water (500 mg/kg)
6.	ZnO NPs coated with herbal extract of *M. prureins*	6	10 ml/ kg Dist water (50 mg/kg)

The following treatments were randomly assigned to six groups of six healthy male Swiss Albino of Wistar Strain rats each as shown in Table 1: The standard test (Clomiphene citrate at 50 mg/kg, *M. prureins* seed extract at 250 mg/kg, and 500 mg/kg each), the infertile group (Ornidazole 200 mg/kg BW, distilled water at 10 mL/kg), the normal control group (distilled water at 10 mL/kg), and the group receiving ZnO nanoparticles coated with herbal extract of *M. prureins* seeds will be 50 mg/kg BW). Feed intake was calculated daily and body weight was recorded weekly.

Feed efficiency ratio was calculated as:

$$FER = \frac{\textbf{weight gain (g)}}{\textbf{feed intake (g)}}.$$

The rats were put to death by extended exposure to diethyl ether anesthesia at the conclusion of the experiment, and heart punctures were used to extract blood samples into sterile centrifuge tubes. The blood was allowed to coagulate for ten minutes before being centrifuged for fifteen minutes at 3000 rpm to separate the serum used to estimate the levels of testosterone, FSH, and LH. Cauda epididymis tails were cut and then gently squeezed onto a clean glass slide in order to extract semen samples. In order to assess epididymal sperm parameters, the semen was employed. Until they were processed for histological analysis, the right testes were kept in a 10% formalin solution.

2.6 Acute Oral toxicity test:

The *M. prureins* seed extract's acute oral toxicity was evaluated using the Organization for Economic Co-operation and Development's (OECD) guideline No. 423 for acute toxic class procedures (OECD, 1996). As advised by the recommendation, female, healthy, nulliparous, and non-pregnant rats were chosen for the investigation. The animals were fasted for three to four hours after the extract was administered, and they were allowed unlimited access to water throughout this time. A single dosage of the extract was given orally at graded concentrations of 2000, 4000, and 5000 mg/kg body weight; each dose level was evaluated in a separate animal. In order to identify any immediate negative effects, the animals were continuously monitored for the first four hours after injection at 30-minute intervals. After then, the rats were observed every day for 14 days to check for death, variations in food and water intake, changes in body weight, and clinical indicators of toxicity, such as tremors, diarrhea, lethargy, paralysis, and motor impairment. The *M. prureins* seed extract was thought to have a broad margin of safety in the lack of fatality or serious toxic symptoms at the maximum dose provided. Consequently, it was deduced that under the experimental conditions, the median lethal dose (LD_{20}) was higher than 5000 mg/kg body weight.

2.7 Infertility induction:

Ornidazole was administered orally to male albino rats at a dose of 200 mg/kg body weight daily for 28 days in a row, with the exception of the control group, in order to induce male infertility. To ensure accurate dosage, the medication was given orally by gavage. Rather than directly causing testicular toxicity, this regimen is known to cause oligoasthenospermia, which is characterized by decreased sperm motility and concentration. This is mainly due to functional impairment of epididymal sperm maturation. Physiological saline was administered in a comparable volume to the control animals (Oberlander et al., 1988).

3. Results:

3.1 Qualitative Phytochemical Screening of 70% Hydroethanolic Extract

Different bioactive secondary metabolites were found in the 70% hydroethanolic seed extract of Mucuna pruriens, according to preliminary phytochemical analysis. Polar and moderately polar components were effectively removed using the hydroethanolic solvent system. All the Qualitative Phytochemical screening results obtained from *M. prureins* seed extract were shown in Table 2 below.

Table 2: Qualitative phytochemical profile of 70% hydroethanolic extracts of Mucuna pruriens seeds

SN	Phytochemical Constituents	Test Performed	Observation	Intensity	Re
1	Alkaloids	Mayer's / Dragendorff's	Creamish precipitate	+++	Pre t
2	Terpenoids	Salkowski test	Reddish-brown ring	++	Pre t
3	Flavonoids	Alkaline reagent test	Yellow coloration	++	Pre t
4	Tannins	Gelatin test	White precipitate	++	Pre t
5	Saponins	Froth test	Persistent foam	++	Pre t
6	Cardiac glycosides	Keller–Killiani test	Brown ring formation	+	Pre t
7	Steroids / Phytosterols	Liebermann–Burchard test	Green coloration	+	Pre t
8	Carbohydrates	Molisch test	Violet ring	+	Pre t
9	Proteins & Amino acids	Ninhydrin test	Purple coloration	+	Pre t
1	Phenolic compounds	Ferric chloride test	Deep blue-green colour	+++	Pre t
1	Anthraquinones	Borntrager's test	No pink colour	–	Ab t

Intensity grading: (+++ = strongly present; ++ = moderately present; + = mildly present; − = absent)

3.2 Particle Size Analysis:

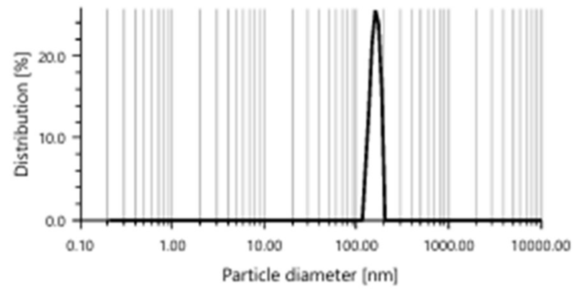

Fig. 2 Peak showing particle size of ZnO Nanoparticles coated with herbal extract of *M. prureins*

Results

Hydrodynamic diameter	149192 nm	Mean intensity	261.5 kcounts/s
Polydispersity index	0.112	Absolute intensity	7985.7 kcounts/s
Diffusion coefficient	0.0 µm²/s	Intercept g1²	0.2120
Transmittance	32.1 %	Baseline	1.031

Particle size distribution peaks (intensity)

Peak name	Size [nm]	Area [%]	Standard deviation [nm]
Peak 1	162.29	100.00	16.52
Peak 2	-	-	-
Peak 3	-	-	-

Fig. 3 Results analysis of ZnO Nanoparticles coated with herbal extract of *M. prureins*

The hydrodynamic particle size distribution of green produced ZnO nanoparticles coated with herbal extract was assessed using dynamic light scattering (DLS) technique. The produced nanoparticles showed a monodispersed particle population in the colloidal suspension, with an average hydrodynamic diameter of 149.192 nm and a significant intensity distribution peak at 162.29 nm that accounted for 100% of the area distribution. The polydispersity index (PDI) that was obtained was 0.112, indicating that the produced nanoparticles had a limited particle size dispersion and good homogeneity as shown in (Figure 2 & 3). The hydration layer and phytochemical coating around the nanoparticle surface may be responsible for the measured hydrodynamic particle size exceeding the typical nanoscale range of 1–100 nm, since DLS measures the hydrodynamic diameter rather than the actual core particle size. By creating an organic corona around the nanoparticle surface, adsorbed biomolecules from plant/herbal extracts greatly improve the hydrodynamic diameter of green produced nanoparticles. Furthermore, the reported particle size may be further increased by a little aggregation of nanoparticles in suspension. Because of the regulated nucleation and development during biosynthesis, the synthesized nanoparticles have a reasonably homogenous size distribution with limited polydispersity, as indicated by the low PDI value found in this study. Generally, PDI values below 0.3 are considered indicative of monodisperse nanoparticle systems, thereby confirming the uniformity and reproducibility of the synthesized ZnO nanoparticles.

3.3 Zeta Potential Analysis

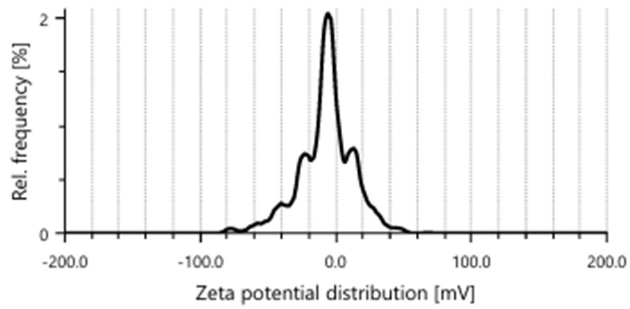

Fig. 4 Peak of zeta potential analysis of ZnO Nanoparticles coated with herbal extract of *M. prureins*

Results

Mean zeta potential	-8.7 mV
Standard deviation	0.8 mV
Distribution peak	-5.4 mV
Electrophoretic Mobility	-0.6804 µm*cm/Vs

Fig. 5 Results analysis of Zeta potential of ZnO Nanoparticles coated with herbal extract of *M. prureins*

The surface charge properties and colloidal stability of the green produced ZnO nanoparticles functionalized with herbal extract were assessed using zeta potential analysis. The produced nanoparticles demonstrated a distribution peak at −5.4 mV and a mean zeta potential value of −8.7 mV, indicating the dominance of negative surface charge on the nanoparticle interface as shown in Figure (4 & 5). The adsorption of phytochemical components from the herbal extract onto the ZnO nanoparticle surface during biosynthesis is responsible for the creation of negative surface potential. Ionizable functional groups that provide surface negativity and aid in nanoparticle stabilization are probably contributed by these phytoconstituents, which include polyphenols, flavonoids, tannins, and other oxygenated biomolecules. The current study's negative zeta potential shows that the phytochemical capping and surface functionalization of ZnO nanoparticles were successful, confirming the herbal extract's function as a stabilizing and reducing agent during synthesis. Similar negative zeta potential values have been reported in the synthesis of ZnO nanoparticles mediated by plants, where stabilizing behavior is greatly influenced by bioorganic molecules adsorbed on the particle surface.

The measured zeta potential magnitude indicates that the produced nanomaterial has modest dispersion stability from the standpoint of colloidal stability.

Zeta potential values within lower ranges indicate less electrostatic stability and increased susceptibility to aggregation, while values above ±30 mV are often thought to indicate extremely stable colloidal systems because of strong electrostatic repulsion. Because there is inadequate repulsive force between neighboring particles, the produced ZnO nanoparticles may display partial agglomeration, as suggested by the significantly reduced magnitude seen here. The intrinsic high surface energy of ZnO nanostructures, which encourages particle–particle interaction and clustering, may be linked to these phenomena. The existence of negatively charged phytochemical moieties adsorbed onto the nanoparticle surface is confirmed by the negative electrophoretic mobility, which shows directed migration of nanoparticles toward the positively charged electrode under an applied electric field.

3.4 Scanning Electron Microscopy (SEM) Analysis

The surface appearance and microstructural properties of the green produced ZnO nanoparticles coated with herbal extract were examined using scanning electron microscopy (SEM). The development of mostly irregularly shaped to quasi-spherical nanoparticles with observable agglomerated cluster formation throughout the sample matrix was visible in the SEM image. After drying, the particles seemed to be dispersed in dense assemblies with moderate interparticle aggregation, suggesting that the nanoparticles were closely packed. The intrinsic high surface energy of ZnO nanoparticles, which encourages particle–particle contact and clustering during drying and sample preparation, may be responsible for the observed agglomerative morphology.

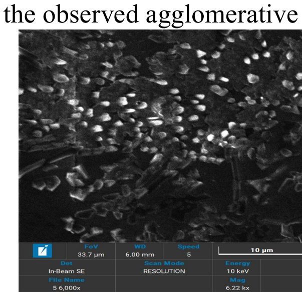

Fig 6. SEM image of ZnO Nanoparticles coated with *M. prureins* Nanoparticles

Additionally, the mild aggregation seen in the SEM image is consistent with the previously determined zeta potential value (−8.7 mV), which indicated a partial agglomeration propensity and limited electrostatic stability of the nanoparticle solution. Additionally, the morphology showed that the nanoparticles have distinct particle boundaries and comparatively consistent nanoscale architecture, indicating that the green production of ZnO nanostructures was successful. The herbal extract's phytochemical adsorption, which serves as a natural capping and stabilizing agent during nanoparticle nucleation and development, may be linked to the modest surface roughness and uneven edges. Biosynthesized nanoparticles exhibit this kind of surface alteration, which further validates the efficient participation of biomolecules originating from plants in the synthesis process.

Furthermore, the hydrodynamic diameter of 149.192 nm found in the dynamic light scattering (DLS) data is in good agreement with the clustered morphology seen through SEM. DLS measures the hydrodynamic diameter of particles in suspension, including the surrounding solvation layer, phytochemical corona, and nanoparticle aggregates, whereas SEM directly visualizes the dried physical particle morphology. This is why DLS yields a relatively larger particle size than SEM. All things considered, the SEM examination validates the effective biosynthesis of ZnO nanoparticles with nanoscale shape and mild agglomeration behavior. The discovered structural characteristics show that the produced material has appropriate morphological features for possible biological, catalytic, and antibacterial applications and are consistent with green generated ZnO nanoparticles described in literature.

3.5 Statistical Analysis: Mean ± standard errors (SE) were used to display the data. The computerized statistical package of social sciences (SPSS) program (SPSS. 20 software version) was used for the statistical analysis. Duncan's multiple range tests were used after one-way analysis of variance (ANOVA).

3.6 Body Weight, Feed Intake and feed efficiency ratio of Experimental rats: In comparison to the control group, ornidazole (OZ) treatment dramatically decreased body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER) (Figures 7, 8, and 9), indicating poor growth performance. Clomiphene citrate treatment resulted in a partial improvement in these metrics. *Mucana prureins* seed extract (MPE) administration considerably and dose-dependently reduced OZ-induced changes; the 500 mg/kg dose was more effective than the 250 mg/kg dose. Rats treated with ZnO nanoparticles mediated by *Mucana prureins* seed extract showed the most noticeable improvement in BWG, FI, and FER, with values that were comparable to those of the control group.

$$\text{BWG} = \frac{\text{Final body weight} - \text{Inital Body Weight}}{\text{number of weeks or defined interval}}$$

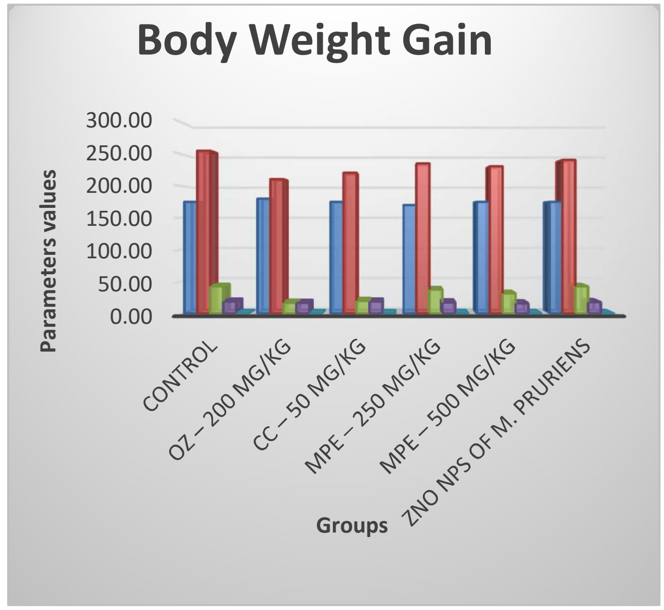

Fig. 7 Effect of *M. prureins* seeds extract and their different dose on body weight gain (BWG)

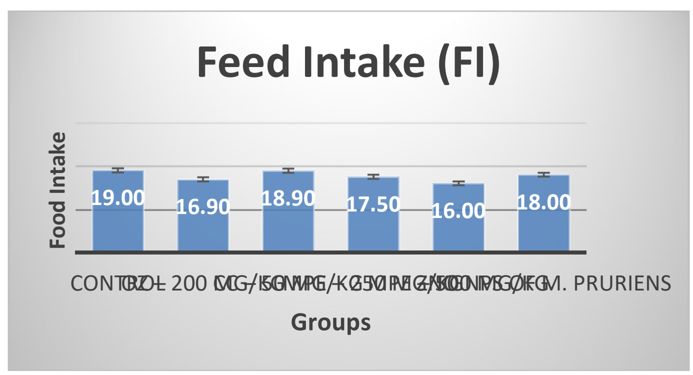

Fig 8. Effect of *M. prureins* seeds extract and their different dose feed intake (FI)

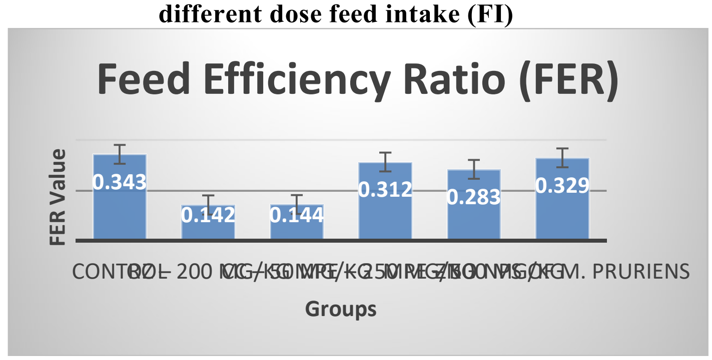

Fig.9 Effect of *M. prureins* seeds extract and their different dose

Ornidazole administration significantly reduced the experimental rats' body weight gain, feed intake, and feed efficiency ratio when compared to the normal control group. The body weight growth and ultimate body weight of rats administered ornidazole alone significantly decreased, indicating reduced food intake and metabolic impairment. On the other hand, mucuna pruriens seed extract treatment shown a protective effect against these alterations. The 250 mg/kg dose improved feed efficiency and body weight gain, suggesting enhanced metabolic activity and the restoration of regular physiological functions. Similarly, growth performance indicators significantly improved using ZnO nanoparticles produced with Mucuna pruriens. These findings imply that the plant extract and its nanoparticle formulation have significant antioxidant and restorative properties that can offset ornidazole's toxicity. The observed improvements could be attributed to bioactive phytoconstituents that increase appetite, digestion, and cellular metabolism in treated animals.

3.7 Impact on Male Relative Weights Reproductive Organs: Compared to the control group, ornidazole (OZ) intoxication significantly reduced the relative weights of seminal vesicles and testes, indicating impaired development of the reproductive organs. (Figure 10).

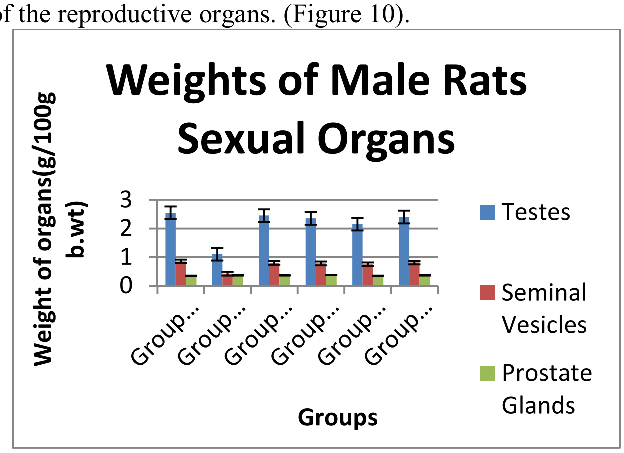

Fig 10. Effect on Relative Weights of Male Reproductive Organs

These losses were considerably lessened by treatment with clomiphene citrate and *Mucana prureins* seeds extract (MPE), with both MPE doses returning organ weights to normal. In terms of testicular and seminal vesicle weights, the ZnO nanoparticle-conjugated *M. prureins* extract showed the best recovery, closely matching control values. On the other hand, there was no discernible difference in the prostate gland's relative weight between the experimental groups.

Following exposure to ornidazole, the relative weight of the testes and seminal vesicles dramatically dropped, indicating impaired androgenic activity and reproductive harm. The reduction in organ weight may be related to spermatogenic activity inhibition and reproductive tissue degeneration. Following treatment with M. pruriens extract, the relative weights of the reproductive organs were significantly and dose-dependently recovered. Testes and seminal vesicle weight significantly improved in the animals treated with the 250 mg/kg extract as compared to the toxic control group. ZnO nanoparticles derived from the plant extract also boosted the weights of reproductive organs, suggesting improved hormonal balance and tissue protection. Interestingly, the weight of the prostate gland remained relatively similar in all groups, indicating that Ornidazole poisoning exclusively affects reproductive tissues. Taking everything into account, the findings show that Mucuna pruriens has potent reproductive protective qualities against chemically induced reproductive failure.

3.8 Effect on Serum Reproductive Hormone Levels: When compared to the control group, ornidazole (OZ) exposure significantly reduced serum levels of testosterone (T), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), suggesting disturbance of the hypothalamic–pituitary–gonadal axis (Figure 11).

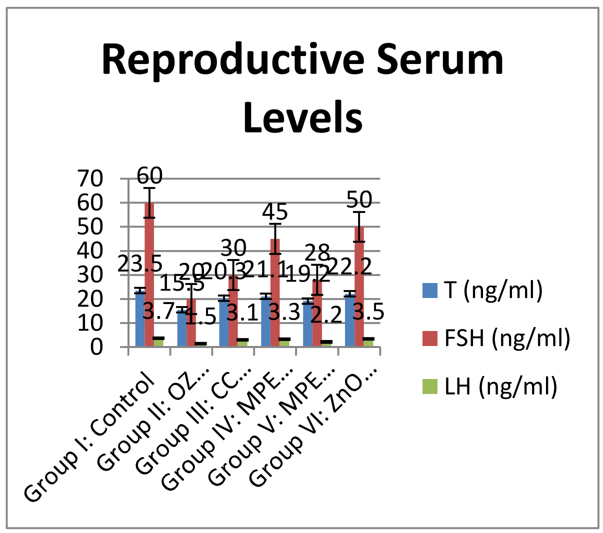

Fig. 11. Serum levels of testosterone (T), follicle stimulating hormone (FSH) and luteinizing hormone (LH)

Treatment with clomiphene citrate and *Mucana prureins* seeds extract (MPE) significantly restored hormone levels; the highest improvement was observed with the higher MPE dose (500 mg/kg). T, FSH, and LH concentrations were most strongly normalized in rats administered ZnO nanoparticles mediated by *Mucana prureins* seed extract (MPE), with levels nearly comparable to those of the control group. When ornidazole was administered, serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone significantly decreased in comparison to the control group, suggesting endocrine dysfunction and compromised reproductive physiology. The drop in hormone levels may be related to oxidative stress-induced damage to the hypothalamic-pituitary-gonadal axis. After using Mucuna pruriens extract, hormone levels were successfully restored to normal. The greater hormonal recovery of the 250 mg/kg dose over the higher dose indicated an optimal therapeutic concentration. Additionally, ZnO nanoparticles derived from the plant extract markedly increased levels of LH, FSH, and testosterone, suggesting improved endocrine control. The hormonal restoration observed in treated groups demonstrates Mucuna pruriens' androgenic and fertility-enhancing qualities. These advantages could be attributed to the availability of naturally occurring phytochemicals, antioxidants, and L-DOPA-like compounds that encourage the production and release of reproductive hormones.

3.9 Semen analysis: The approach outlined by Bearden and Fluquary (Bearden HJ et al., 1980) was used to determine the sperm progressive motility and count. According to (Amann et al., 1982), microscopic analyses of the seminal smears stained with Eosin Nigrosin stain were also performed to ascertain the percentages of sperm viability and abnormalities. In comparison to the control group, ornidazole (OZ) poisoning resulted in a considerable decline in sperm count, motility, and viability as well as a marked rise in sperm abnormalities(Figure 12).

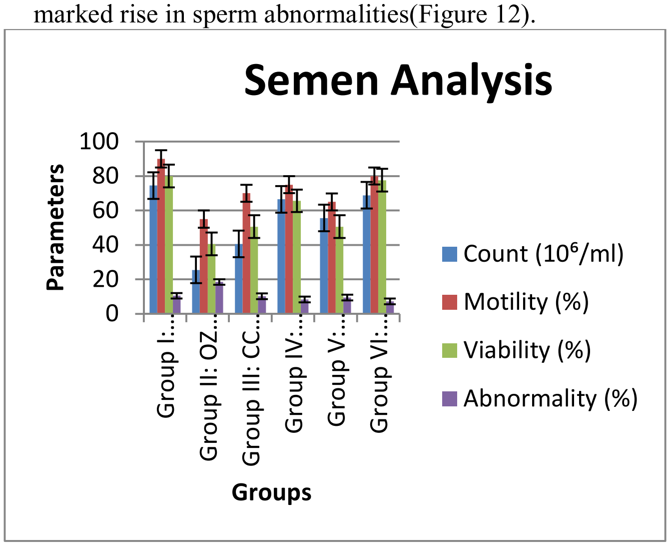

Fig. 12 Effects of Semen Quality in different groups

These parameters were somewhat improved by clomiphene citrate treatment, but the results were still much lower than those of the control group. In a dosage-dependent manner, administration of *Mucana prureins* seed extract (MPE) considerably reduced OZ-induced sperm failure; the 250 mg/kg dose was more effective than the 500 mg/kg dose. Notably, rats given ZnO nanoparticles mediated by *M. prureins* extract showed the greatest improvement in sperm count, motility, and viability as well as a significant decrease in sperm abnormalities, with values that were very similar to normal control levels. According to the semen study, ornidazole prescription significantly decreased sperm count, motility, and viability while increasing sperm abnormalities, indicating its negative effects on male reproductive health. Lower-quality sperm can result from oxidative stress, lipid peroxidation, and damage to testicular cells. Sperm parameters were enhanced when treated mice were given Mucuna pruriens extract. The 250 mg/kg dose markedly improved sperm count, motility, and viability while also reducing the percentage of faulty sperm. Furthermore, semen qualities significantly improved with ZnO nanoparticles derived from the plant extract, approaching normal control values. These findings suggest that the extract contains antioxidant and spermatogenic properties that protect germ cells from injury. The bioactive ingredients in Mucuna pruriens may enhance reproductive success by promoting healthy sperm production, reducing oxidative stress, and stabilizing cellular membranes.

3.10 Histopathology of Testis:

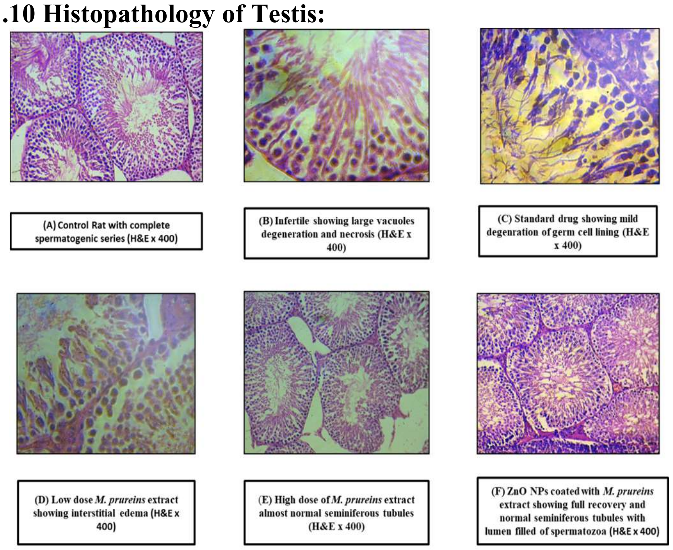

FIGURE 7: Photomicrographs representing H&E-stained testicular tissue sections of the control groups. (A) control (B) Ornidazole , (C) Clomiphene citrate , (D & E) Low and High dose of *M. prureins* (F) ZnO NP's Mediated with *M. prureins* . All these groups displayed typical testicular structures.

After that, the fixed testicular specimens were cut, cleaned, and dehydrated in increasing alcohol concentrations. After being embedded in paraffin, these samples were sectioned at a thickness of 4–6 microns, stained with hematoxylin and eosin (H&E), and then inspected under a microscope. Testicular tissue from the normal control group showed well-preserved seminiferous tubules with an intact germinal epithelium and a full and well-organized spermatogenic sequence, according to histological analysis.

4. **Discussion:** The current results show that secondary metabolites, especially phenolic and alkaloids, are abundant

in the 70% hydroethanolic extract of Mucuna pruriens seeds. The presence of L-DOPA, the main pharmacologically active substance with neuroprotective and anti-Parkinsonian effects, is consistent with the robust alkaloid reaction. Nine verified types of bioactive secondary metabolites were included in the extract, confirming its therapeutic significance and bolstering its use in pharmacological and green nanoparticle manufacturing research. Scanning electron microscopy (SEM), dynamic light scattering (DLS), and zeta potential analysis were used to thoroughly physicochemically characterize the green produced ZnO nanoparticles in order to assess their shape, particle size distribution, and colloidal stability. Throughout the sample matrix, SEM analysis showed the production of mostly irregular to quasi-spherical ZnO nanoparticles with moderate aggregation and clustered shape. Because of their high surface energy and inherent propensity for particle-particle contact during drying and storage, ZnO nanostructures exhibit the observed agglomeration. DLS measurements revealed a strong intensity peak at 162.29 nm and an average hydrodynamic diameter of 149.192 nm, indicating a distribution of nanoscale particles within the colloidal suspension. Due to the hydrodynamic nature of DLS measurement, which takes into account the solvation layer, phytochemical coating, and agglomerated nanoparticle clusters present in suspension, the particle size measured from DLS was significantly bigger than that observed in SEM. This increase in hydrodynamic diameter is frequently linked to the adsorption of biomolecular capping agents from the herbal extract onto the surface of green produced nanoparticles.

Additionally, the produced ZnO nanoparticles showed a zeta potential of −8.7 mV, confirming successful phytochemical functionalization and suggesting the presence of a negatively charged nanoparticle surface. The effective synthesis of herbal extract-mediated ZnO nanoparticles with nanoscale morphology, phytochemical surface capping, and a mild aggregation propensity is confirmed by the correlation between SEM, DLS, and zeta potential data. These results confirm the green synthesis approach's efficacy and imply that the produced nanoparticles have suitable physicochemical characteristics for additional biomedical and antibacterial research.

Interestingly, concurrent oral treatment of zinc oxide nanoparticles (ZnO NPs) coated with extract from *M. prureins* seeds significantly reduced reproductive harm caused by OZ. Reproductive organ weights were significantly restored, sperm count, motility, and viability improved, and serum reproductive hormone levels returned to normal after treatment with the nano-formulated herbal extract. Furthermore, testicular antioxidant enzyme activities were greatly increased by the coated ZnO NPs, which decreased oxidative burden and limited cellular damage caused by lipid peroxidation. Histological findings further supported the decrease of necrotic and degenerative lesions and the partial preservation of testicular architecture. The increased bioavailability, higher cellular absorption, and synergistic interactions between zinc and the phytoconstituents of *M. prureins*, such as polyphenols and flavonoids, may be responsible for the superior protective performance of ZnO NPs coated with *M. prureins* as compared to the crude extract alone. While antioxidants derived from plants aid in cytoprotection and free radical scavenging, zinc is recognized to be essential for spermatogenesis, testosterone production, and testicular redox equilibrium. When taken as a whole, these actions may support the stabilization of the testicular microenvironment, the maintenance of germ cell viability, and the restoration of steroidogenesis.

5. **Conclusion:** The current study concludes that Mucuna pruriens' 70% hydroethanolic seed extract is a rich source of physiologically active phytoconstituents, including alkaloids, phenolics, and flavonoids, which support the plant's strong antioxidant and reproductive protecting qualities. SEM, DLS, and zeta potential investigations verified the successful green production of zinc oxide nanoparticles using the seed extract, exhibiting nanosized particles with adequate colloidal stability and phytochemical surface capping. The created nanoformulation is appropriate for biomedical applications, according to these physicochemical properties. Treatment with M. pruriens-coated zinc oxide nanoparticles considerably increased endogenous antioxidant defenses, regulated serum reproductive hormones, improved sperm quality, and recovered reproductive organ weights in the olanzapine-induced model of male reproductive toxicity. Histopathological analysis also showed a significant reduction in degenerative lesions and preservation of testicular architecture. Improved bioavailability and synergistic interactions between zinc and plant-derived antioxidants are suggested by the nanoformulation's higher efficacy compared to the crude extract alone. Spermatogenesis, steroidogenesis, and redox equilibrium are all aided by zinc, and phytoconstituents have potent cytoprotective and free radical scavenging properties. When combined, these processes support the stabilization of the testicular microenvironment and the restoration of germ cell integrity. The results show that M. pruriens-mediated ZnO nanoparticles are a promising nanotherapeutic approach for reducing male infertility linked to oxidative stress. The benefits of herbal medicine and nanotechnology are combined in an environmentally friendly synthetic formulation to improve therapeutic efficacy. To promote its clinical translation as a novel treatment for male reproductive problems, more research on molecular processes, pharmacokinetics, and long-term safety evaluation is necessary.

Declaration:

Ethical Consideration- All experimental procedures involving animals were conducted in strict accordance with the internationally accepted guidelines for the care and use of laboratory animals, as outlined in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH, 2011). Prior to the initiation of the experimental study, the experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of NIMS University Rajasthan, Jaipur, under approval number NIMSUR/IAEC-01/2024/05. All efforts were made to minimize animal suffering and to reduce the number of animals used throughout the experimental procedures.

Conflict of Interest Statement- The authors have no conflicts of interest to declare for this study.

Data availability statement- All data and materials for this study will be provided upon request to the corresponding author.

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Contribution Statement with Credit Authorship:
Ms. Princy Kumari Rajput (Research scholar) has contributed to study conception and design, material preparation, data collection and compilation of experimental data. Dr. Priyanka Singh (as supervisor) has guided for designing of experiment, analysis of data and drafting of manuscripts. Dr. Yogesh Kumar Singh has helped for therapeutic efficacy of plant extract and nanoparticle for In vivo study in Rat model. All authors have read and approved the final manuscript.

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