RESEARCH ARTICLE

Green Synthesis of Zinc Nanoparticle of Seed Extract of Fenugreek and Pumpkin

Alshifa Memon[1], Yogesh Kumar Singh[2], Priyanka Singh[3*]

[1]*PhD Scholar, Department of Zoology, Nims Institute of Allied Medical Science and Technology, Nims University Rajasthan, Jaipur, India, 303121*
[2]*Advance Molecular Laboratory, Nims University Rajasthan, Jaipur, India, 303121*
[3]*Departmen of Biotechnology, Nims institute of Allied Medical Science and Technology, Nims University Rajasthan, Jaipur, India, 303121*

Corresponding Author: Priyanka Singh*

Received: 22th May, 2026; Revised: 6th June, 2026; Accepted: 8th June, 2026; Available Online: 09th June, 2026

ABSTRACT
Green synthesis of nanoparticles has emerged as an eco-friendly and sustainable alternative to conventional chemical methods. In this study, zinc nanoparticles (ZnNPs) were synthesized using seed extracts of fenugreek (Trigonella foenum-graecum) and pumpkin (Cucurbita pepo) as reducing and stabilizing agents. The bioactive compounds present in these seed extracts, such as flavonoids, phenolics, and proteins, play a crucial role in the reduction of zinc ions and stabilization of the formed nanoparticles. The synthesis process was carried out under ambient conditions without the use of toxic chemicals, making it environmentally benign. The formation of zinc nanoparticles was confirmed through observable color change and further characterized using techniques such as UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). The results indicated the successful formation of stable, predominantly spherical nanoparticles with nanoscale dimensions. Furthermore, the synthesized ZnNPs were evaluated for their biological activities, including antioxidant and antimicrobial properties. The nanoparticles exhibited significant activity against selected microbial strains, suggesting their potential application in pharmaceutical and biomedical fields. This study demonstrates that fenugreek and pumpkin seed extracts are effective, low-cost, and sustainable resources for the green synthesis of zinc nanoparticles with promising functional properties.
Keywords: Zinc Nanoparticle, Pumpkin seed, Fenugreek seed
How to cite this article: Alshifa Memon, Yogesh Kumar Singh, Priyanka Singh. Green Synthesis of Zinc Nanoparticle of Seed Extract of Fenugreek and Pumpkin. International Journal of Drug Delivery Technology. 2026;16(79s): 43-59. DOI: 10.25258/ijddt.16.79s.6
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Male azoospermia is the total lack of the spermatozoa in ejaculate and one of the major causes of male infertility in humans and most of the animal species. It is no disease, but is a clinical picture that is indicative of dysfunction in sperm production, sperm carriage, hormones, or reproductive anatomy. Azoospermia has attracted medical as well as zoological interest where it has been shown that the male reproductive system is a very delicate and highly coordinated system that even requires only a few cells to generate an impressive effect. It has an impact on natural reproduction, breeding success, and population sustainability, so it is not only of interest to the human fertility clinics (Jarow et al., 1989). Azoospermia is approximated in 1 percent of all men in human medicine, and in 10-15 percent of infertile men. This causes it to be a rather rare case within a general population, but a significant concern to couples experiencing infertility. The process of diagnosis usually comes at a very unlikely time when the semen seems normal after normal semen is a very sensitive process and therefore, azoospermia is commonly an indication of more general reproductive insufficiency (O'Donnell, Stanton, and de Kretser, 2001). In most instances, genetic influences greatly contribute to male azoospermia. Severe spermatogenic failure is widely related to chromosomal abnormalities like Klinefelter syndrome, deletions of the Y chromosome microdeletions, and mutations of testicular formation. These discoveries have revolutionized the research in terms of infertility because they have indicated that genetic architecture's role in reproduction is intricate. Comparative evolutionary studies of chromosomal infertility have also been used to explain the evolution of reproductive barriers between species in zoology (Krausz & Casamonti, 2017). Another significant cause of azoospermia is hormonal disturbances. Conditions associated with hypothalamus, pituitary gland or testes may disrupt the endocrine signals needed in the production of sperm. It may be due to low secretion of gonadotropin, hyperprolactinemia, androgen deficiency, and thyroid dysfunction. Some of these causes are correctable in humans hence endocrine analysis is a very crucial element of diagnosis. Hormone-linked infertility patterns like these have been seen in captive and domestic animals subjected to stress or inadequately fed (McLachlan,

**Author for Correspondence: priyay20@gmail.com*

O'Donnell, Meachem, Stanton, and de Kretser, 2002). Environmental exposure is also an emerging issue of interest in azoospermia studies. Testicular function can be damaged by heat stress, pesticides, heavy metals, air pollution, endocrine-disrupting chemicals and radiations. People who live in the industrial or highly polluted locations are usually associated with a lower quality of semen. Experienced effects of environmental toxicants include declined reproductive function in fish, reptiles, birds and mammals in the field of wildlife biology. Azoospermia thus is not just a personal health problem, but also a bioindicator of an ecological imbalance (Sharpe & Irvine, 2004). Lifestyle factors also play a role in risk of azoospermia or severe sperm suppression. Hormonal imbalance or a simple destruction of spermatogenesis can be caused by smoking, alcohol abuse, using anabolic steroids, obesity, chronic stress, poor sleep, and substance misuse. Although all men who are exposed may not turn into azoospermic, these aspects can aggravate pre-existing susceptibilities. Human research is progressively relating reproductive health and general metabolic health as well as behavioral health and it has been seen that fertility is a reflection of a greater physiological health (Palmer et al., 2012). Azoospermia also has significant implications in the production of livestock in the context of zoological studies. The males of the stallion breed of poultry and the breeding males of bulls, rams, boars and stallions should have high fertility. In case of azoospermia, this may cause massive losses on the economic front due to breeding unsuccessful schemes and lower genetic advancement. The veterinary reproductive experts thus examine the quality of semen, endocrine condition, infections and genetic defects to ensure breeding efficiency (Hafez & Hafez, 2000). Azoospermia is becoming an issue of concern with companion animals such as dogs and cats being taken to look after their breeding viability. Reasons could be borchitis, trauma, testicular degeneration, a birth defect, or age-related degeneration. Since selective breeding tends to decrease genetic variety, inherited reproductive errors may turn out to be more misunderstood within a particular range of descent. This has resulted in companion animal fertility being a significant area bridging veterinary medicine, genetics and animal welfare (Johnston, Root Kustritz and Olson, 2001). Another aspect of azoospermia is wildlife conservation. Even infertility in a limited number of breeding males in endangered species with an already small population is likely to have a great impact on recovery. Seminemia analysis and assisted reproduction are common in captive breeding programs of large cats, primates, ungulates and rare birds. In case of azoospermia, managers might be required to change making of insemination plans or go to genetic resource banking. Reproductive biology is thereby directly utilized in the management of conservation (Wildt, 2005). Temporal azoospermia-like conditions are interesting zoological paradigms through seasonal breeders. Due to natural variations in photoperiod, temperature and breeding season, sperm production increases and decreases in some mammals, birds, and reptiles. Non-breeding also results in regression of testes and absence of sperms in ejaculates. This is not a pathological but a physiological azoospermia whose investigation aids in informing the scientists about endocrine regulation of fertility and reversible inhibition of spermatogenesis (Lincoln, Short, and Setchell, 1981). The other known cause of azoospermia in humans and other animals is infectious disease. Testicular tissue impairment or sperm duct blockage may occur in men with mumps orchitis, sexually transmitted diseases, tuberculosis or post-inflammatory scarring. In animals, fertility could be decreased by brucellosis, viral orchitis, bacterial epididymitis, and parasitic infections. These illustrations reveal that the aspect of reproductive failure mostly crosses with immunology and disease ecology (Nieschlag & Behre, 2010). The application of the new diagnostic techniques has completely enhanced literature on azoospermia. The analysis of the semen is requested at the first stage, but in our case, a lot could be learned through the assistance of the hormonal analysis, scrotal ultrasonography, genetic analysis, testicular biopsy and the molecular profiling. More commonly used in animals are ultrasound imaging, semen collection technologies, histopathology, and implementation of genomic tools. The techniques enable the scientists to differentiate reversible dysfunction and irreversible dysfunction as well as irrevocable germ cell failure more precisely (Esteves et al., 2011). The assisted reproductive technologies have helped azoospermic men have a dark future. Direct retrieval of sperm is performed Intracytoplasmic sperm injection (ICSI) necessitates the direct retrieval of sperm that has been harvested directly at the testes or in a few cases is directly retrieved at the epididymis and in any case has already resulted in biological fatherhood of those whom it had previously been considered impossible to treat. Semina preservation, artificial insemination and embryo transfer (in animal science) is used to help boost male infertility, or to derive genetic material that could later be valuable, or to exploit genetic material. The use of these technologies has not only revolutionized the fertility medicine, but also the animal breeding system (Schlegel, 2004). The cellular research has been conducted wherein the azoospermia has been used as a tool to study the stem cell and germ cell biology. The production of sperms is sustained by the spermatogonial stem cells. Destruction of these cells can be done either through toxins, or through genetics or chemotherapy hence leading to permanent infertility. They are under regenerative treatment and they are experimented with proper transplanted stem cell identification in rodents and livestock which have provided an opening in future possibilities about restoring fertility (Brinster, 2007). Another human situation that is critical is in treatment of cancer. Some processes, radiotherapy and chemotherapy may give an azoospermia effect or a temporary and permanent effect on the weapons that are used to destroy the rapidly multiplying germ cells. This has seen a better focus in fertility preservation during the pre-treatment period particularly in sperm banking among adolescents as well as adults. This can be extended to breeding animals of great value that need medication. The issue is linked to oncology and long-term reproduction and the quality of life ethics (Howell and Shalet, 2005). The problem of azoospermia in an evolutionary setting would enable the researcher to test how fertility failure is caused despite the high rate of fertility selecting. The mutation has not disappeared, there are environmental stressors, and reproduction outcomes might be mediated by trade-offs between survival or reproduction. As a by-product of irreverent selection, artificially selected reproductive disorders in the captive population can increase.

Azoospermia-infertile is such a case where comparative zoology can be effectively applied to gain meaningful insights on the practice of life-history, and reproductive customization across species (Birkhead, Hosken, and Pitnick, 2009). The issue of ethics in the study of azoospermia is also a matter. In humans, there are problematic cases related to contributions to donor sperm, recovery and testing of surgeries and reveling of inherited risks. Part of the ethical issues on animals are selective breeding, invasive breeding, manipulation of reproductive processes of its endangered species etc. Conscientious research means that there is a trade-off between scientific progress and health, autonomy and genetic health in future (Wildt, 2005). In general, there is much more to male azoospermia than afebrile of sperm in the semen. It is a multifaceted biological disease, touching on endocrinology, genetics, anatomy, ecology, veterinary science and assisted reproduction. In men, it determines personal problems of sexuality and healthcare. It affects fields of agriculture, biodiversity, conservation and even evolution of animals. Due to this broad applicability, azoospermia has been an influential subject in zoological and biomedical studies and has provided an understanding of how effective and ineffective reproduction unfolds, and a hint of how it might be cured and regenerated in the future (Krausch & Casamonti, 2017).

Male infertility is one of the significant challenges affecting reproductive health on the global level, and azoospermia is considered to be one of the most serious forms of male-factor infertility (Agarwal et al., 2021). Azoospermia is described as no spermatozoa in the ejaculate and it is usually divided into obstructive and non-obstructive (Kumar and Singh, 2015). It is correlated with emotional, social and clinical burdens in human beings, and breeding efficiency, livestock economics and conservation success in animals. Animal models are very commonly employed to comprehend the pathophysiology of impaired spermatogenesis and infertile conditions in the zoological and comparative reproductive research. The effectiveness of rodents, rabbits, poultry, and domestic livestock as models to study testicular degeneration, endocrine imbalance and nutritional interventions have been useful (Durairajanayagam, 2018). They are models that are used to bridge the gap between these laboratory findings and that of human reproductive medicine. The seeds of fenugreek (Trigonella foenum-graecum) are well-known and renowned in terms of their medicinal and nutritional contribution. They are also rich in steroidal saponins, flavonoids, alkaloids, proteins, and minerals that could be

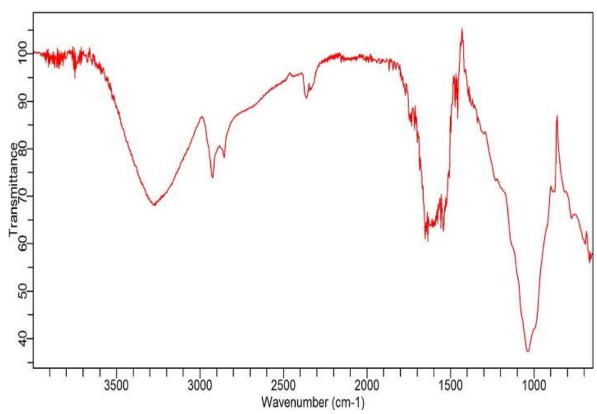

Figure 1. FTIR Analysis of pumpkin seeds crude sample

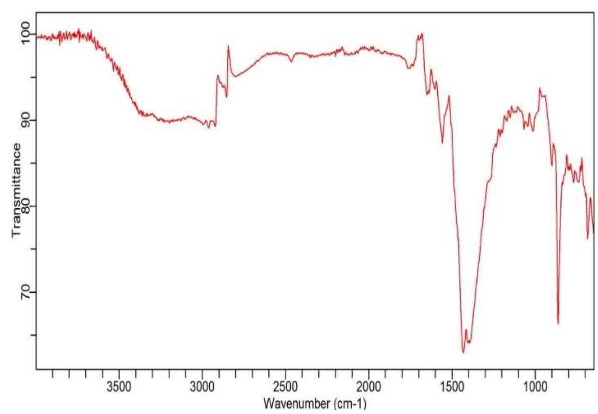

Figure 2. FTIR analysis pumpkin seeds zinc nanoparticles

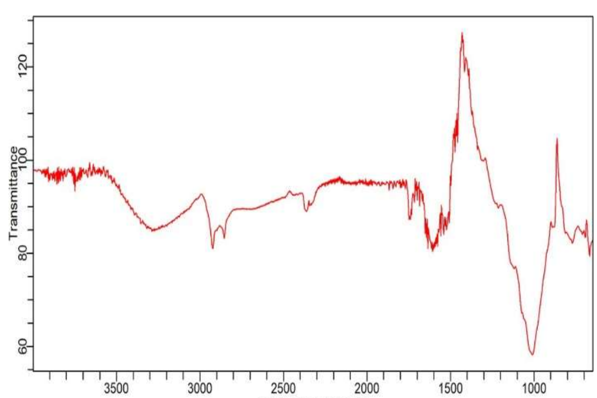

Figure 3. FTIR analysis of mixture of pumpkin seeds and fenugreek leaves crude sample

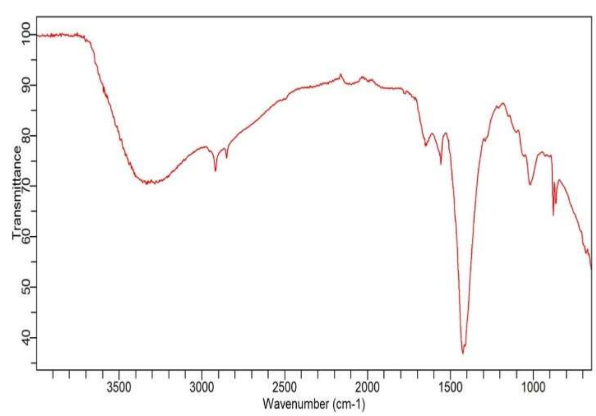

Figure 4. FTIR analysis of Combination of pumpkin seeds and fenugreek seeds zinc nanoparticles

beneficial to reproductive physiology (Wankhede et al., 2016). One of the phytochemicals formed in the process of glycolysis in fenugreek, diosgenin has been associated with hormonal regulation and enhanced androgenicity. A number of studies indicate that the use of fenugreek supplement can potentially enhance testosterone levels, libido, antioxidant conditions, and semen specifics (Wankhede et al., 2016). Fenugreek has also shown to protect against the oxidative damage in reproductive tissues in experimental animals (El-Sayed & Aly, 2015). These results put fenugreek as a fascinating prospect in the azoospermia study. The other valuable functional food is pumpkin seeds (Cucurbita pepo) that contain zinc, selenium, vitamin E, phytosterols, and unsaturated fatty acids (Saeed et al., 2014). These nutrients play a vital role in the health of males since they are involved in the integrity of membranes, hormones production, and antioxidant defense mechanisms.

Animal experiments indicate that the pumpkin seed extract results in protective effects on the reproductive tissues against the adverse conditions of toxicity or oxidative stress conditions (Al-Okbi et al., 2019). This is especially pertinent due to the high levels of zinc, as the lack of zinc was also related to poor spermatogenesis and low semen quality (Yousef et al., 2019). It is possible that the synergistic reproductive effect of the combination of the seeds of fenugreek and pumpkin may give synergistic effects. Fenugreek has endocrine and metabolic benefits and pumpkin seeds are good sources of micronutrients and lipid based antioxidants. They can also be used together to establish a desirable internal microclimate to generate sperm and recovery of the testicles. One of the primary causes of male infertility and azoospermia is believed to be oxidative stress. Abnormal accumulation of reactive oxygen species may cause harm to germ cells, Sertoli cells, Leydig cells and sperm DNA, eventually inhibiting spermatogenesis (Shokri et al., 2020). Thus, seed mixtures that contain antioxidants are scientifically pertinent in managing infertility. Another significant cause associated with dysfunction of the reproductive system is inflammation. The inflammation may be permanent and cause disruption of the blood-testis barrier to induce apoptosis of developing germ cells. Fenugreek and pumpkin seeds contain polyphenols and tocopherols, which could protect against inflammatory damage and maintain the integrity of seminiferous tubules (Aitken and Baker, 2020). Zinc is one of the most important trace minerals to the male fertility. It interferes with testosterone metabolism, DNA replication, stabilization of chromatin, antioxidant enzyme activities, and sperm membrane integrity (Yosef et al., 2019). Low semen parameters and infertility has been frequently connected with low level of zinc. Annual nanotechnology has introduced a novel type of a superior supply of zinc referred to as zinc nanoparticles. The zinc nanoparticles might be more bioavailable and be easily taken up by cells than its conventional particle, zinc salts, due to its small size (molar mass) and great surface area (moghadam et al., 2012). Reproductive biology Zinc nanoparticles in research, Zinc nanoparticles are under investigation to alleviate oxidative stress damage, estrogen levels, and repair testicular damage in test animals. they are capable of increasing the spermatogenic recovery and cell metabolism using controlled doses. Pumpkin seedsdried in zinc nanoparticles and fenugreek seeds kicked are a new way of using nutraceutical technology. The seeds contain the phytochemicals and antioxidants and natural nutrients, and zinc nanoparticles can enhance the targeted delivery of zinc. Such a multifactorial approach will prove to be useful specifically in such situations as non-obstructive

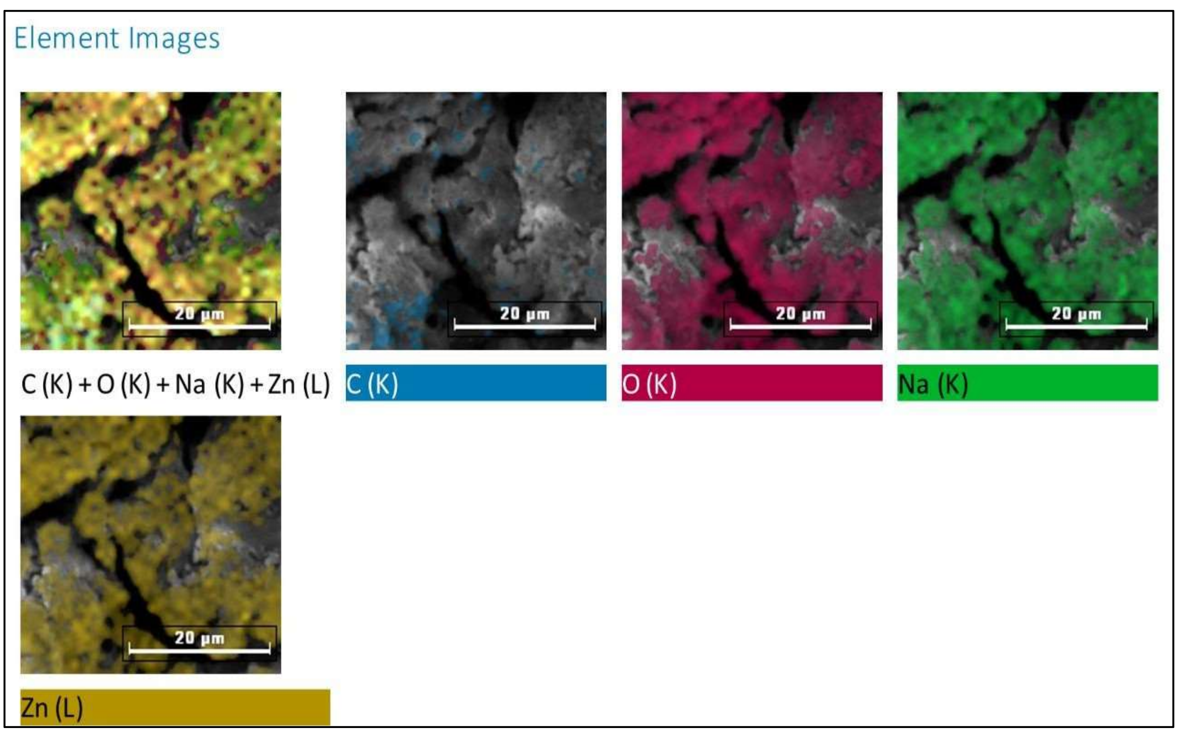

Figure 5. Fesem images of zinc nanoparticle of pumpkin seed extract

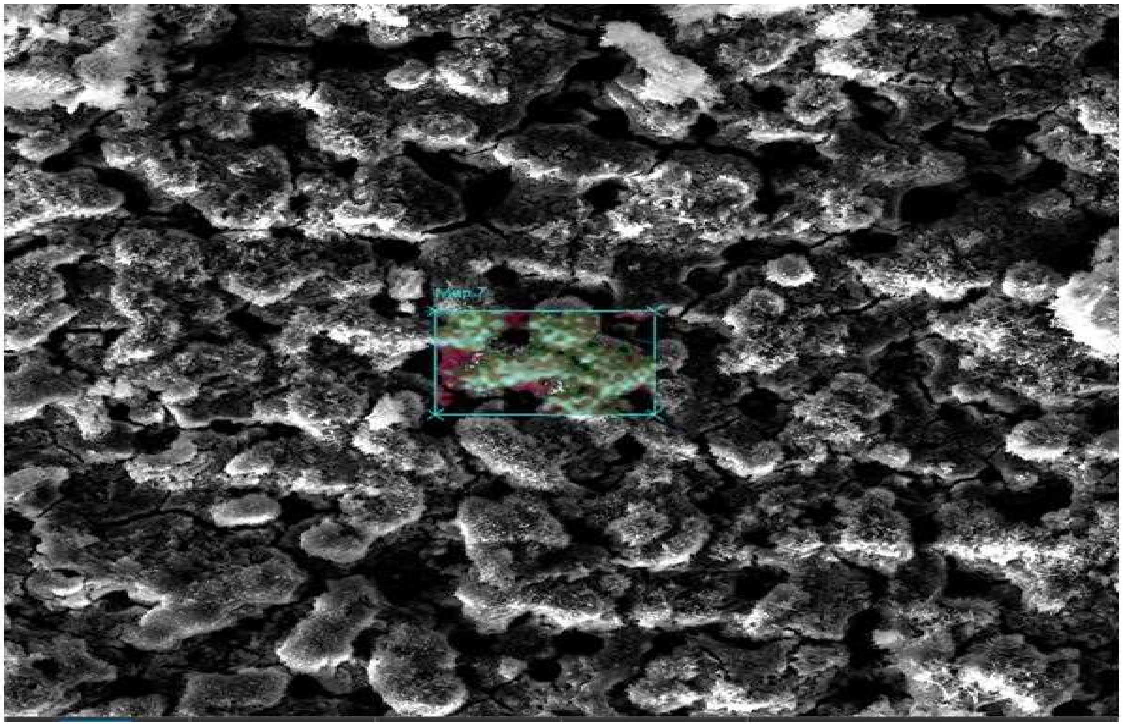

Figure 6. Morphological analysis of zinc nanoparticle by SEM

azoospermia. The research is generally applicable because it can be experimented on animals.

By using heat stress or toxic chemicals or diabetes or pesticide or chemotherapy and testing how well a combination treatment can fix fertility indicators, investigators can induce azoospermia to test it (Durairairajanayagam, 2018). Applicable zoological endpoints include the weight of testicular, seminiferous tube size, number of sperm progenitors, existence of epididymal sperms, level of testosterone and the activity of anti-oxidant enzyme. An upsurge in such indicators would indicate a good recovery of reproduction. The histological analysis should be performed too. The development of spermatogonia, spermatocytes, spermatids as well as the complete spermatozoa in seminiferous tubules would strongly prove that treatment assists in actual spermatogenesis rather than hormonal stimulation alone.

Infertility has a direct negative effects on productivity in livestock such as bulls, rams, bucks and boars. Zinc nanoparticle-enriched fenugreek-pumpkin seed supplements, so long as their safety is ensured, also have the promise of becoming cost-effective feed supplements with the ability to breed males. Reproductive support techniques are gaining significance in captive breeding in wildlife conservation. There are nutritional formulations which can enhance the health of the semen and endocrine which can help the threatened species though they need species-specific testing. In the case of human medicine, such combinations should be regarded as supportive treatments but not an alternative to the diagnosis and conventional treatment. There are several treatment approaches needed in obstructive azoospermia, unlike non-obstructive ones, making patient assessment key (Kumar & Singh, 2015). To sum up, the synergy of the combination of the fenugreek seed varieties and the pumpkin seed in the case of zinc nanoparticles provides an opportunity of zoological and biomedical study on the male azoospermia. This combined solution could be used in future fertility treatments in humans and animals through antioxidant protection, endocrine support, delivery of micronutrients and potential fertility restoration through spermatogenesis restoration.

MATERIALS AND METHODS

Collection of Plant Material

The botanical garden in NIMS University was used to extract fresh seeds of fenugreek (Trigonella foenum-graecum) and pumpkin (Cucurbita pepo) that were harvested and transported to the laboratory where they would be later used during the test. The seeds were then picked and the seeds examined critically which eliminated the damaged or undesirable parts of the seeds. This was followed by thoroughly washing them in a lot of distilled water to dissolve dust, soil particles and any other dirt on the surface. Then the seeds were washed and then placed in a standard distance on clean trays and left to dry in a natural process under an environment of natural room temperatures, as well as in a shady environment. They dried up until all moisture has been evaporated and the seeds remain still in their good condition until it goes through the other storage steps of processing and extraction.The botanicality and authenticity of the plant materials which were collected were also confirmed by Dr. Priyanka Singh. This has been put to trial in the Department of Allied medical science, NIMS University, Jaipur, Rajasthan, India.

This confidence was used to determine the selection of the appropriate plant species to be used in the ongoing study.

Preparation of seeds extract

Plant extract was prepared using the seeds of Trigonella foenum-graecum L. as well as Cucurbita pepo. The seeds collected were dried appropriately under the right conditions and then ground in a clean grinder to fine view to a homogeneous powder in a fine coarse manner. The powder was measured and put into airtight containers in a refrigerated system to maintain the natural phytochemical constituents that remained in the prepared powder up to the next usage. To prepare the extracts, five grams of mixed powdered seed sample were weighed precisely and put in a clean flask with dilute ethanol. This solution was then left to settle at room temperature in 24 hours to enable good extraction of the bioactive compounds present in the seeds. In this time, the solvent took its time and infiltrated the plant material and dissolved the useful phytochemicals that would be used in future in the manufacture of the nanoparticle. Once extraction process was complete, it was filtered using Whatman No. 1 filter paper to get the liquid extract and the solid residues. The filtrate was clear and was collected in a sterile container. The filtrate then obtained was refrigerated at 4 o C to store carefully to be used later in the process of green synthesis of zinc oxide (ZnO) nanoparticles.

Preparation of zinc nanoparticles

To prepare the green synthesis of zinc oxide nanoparticles, zinc acetate (0.1 M) was weighed correctly and 100 mL of double-distilled and weighed in 100 mL of the nanoparticle. The solution was gently stirredary (with constant magnetic stirring) over 10 minutes at room temperature until a clear and homogeneous solution was acquired. This first step allowed the correct dissolution of the zinc precursor, then continued to react.The solution of zinc acetate was mixed thoroughly and with continual stirring, 2 g of sodium hydroxide was gradually added to it. To ensure there was no precipitation and to ensure that the reaction conditions remained constant, the sodium hydroxide was gradually added.After this, dropwise the 5 ml of the prepared mixed seed extract of pumpkin (Cucurbita pepo) and fenugreek (Trigonella foenum-graecum) were added into the reaction

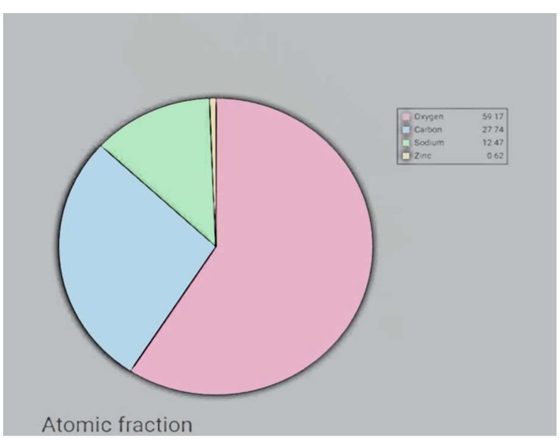

Figure 7. Energy dispersive x-ray spectroscopy (EDX) spectrum

mixture. The solution that was obtained was left to react after 2 hours of continuous stirring at room temperature.

The phytochemical compounds that occurred in the seed extract during this step assumed the roles of natural reducing and stabilizing agents that contributed to the creation of zinc oxide nanoparticles.The reaction mixture pH was kept at 12 with a lot of care since alkaline environment is conducive to the formation of the nanoparticles and it enhances the precipitation process. The pH was continuously monitored to assure consistency and reproducibility of the synthesis.The mixture was centrifuged at the rate of 6000 rpm and 30 minutes were taken following the completion of the period of reaction to separate the formed nanoparticles in the mixture and the supernatant liquid. The material that was precipitated was gathered and was further processed.The resulting product was next heated and boiled by 60 minutes where the outlook of the solution slowly turned into a light white paste and this was to show the formation and concentration of the nanoparticles.A careful transfer of the resulting paste into a clean ceramic crucible was then placed in furnace that was heated by air at 400C to be calcinated. Various time durations (30, 60, 120 and 240 minutes) were used to examine the impact of heating time on the formation and crystallinity of nanoparticles.A light white colored fine powder was obtained after the treatment of the furnace, which proved the successful synthesis of Zinc oxide nanoparticles. The powder was then carefully picked, placed in sterile air tight bags and stored to be characterized further through studies.A last step entailed drying the material and performing some light pounding on it using a mortar and pestle to get a finer and more homogenous powder. This fine texture was needed in order to make accurate and reliable characterization through different analytical methods.

Characterization

FTIR analysis

Fourier Transform Infrared (FT-IR) spectroscopy was performed to reveal and explain the predominant functional groups in both the dried sample of the pumpkin seeds, and the functional groups in the pumpkin seed + fenugreek seed sample which might be involved in the production and stabilization of zinc oxide nanoparticles (ZnO-NPs). FT-IR is an extremely powerful analysis method of determining chemical bonds, and characterizing biomolecules by their vibrational frequencies. It is also commonly used in the field of nanoparticles to identify the contribution of plant phytochemicals in green synthesis (Stuart, 2004). FT-IR standard instrumentation was used to conduct the analysis in the Pharmacy Institute, NIMS University, Jaipur, Rajasthan. FT-IR spectra were taken in transmittance mode in the range of wave numbers 4000-400 cm -1 which is thought to be the most effective range to identify both organic and inorganic functional groups. This range enables the identification of absorption peaks that represent hydroxyl, amine, alkane bonds, aldehyde and ketone bonds, as well as ester, ethers and metal-oxygen bonds. This information is very effective in studying chemical structure of plant materials and their reactions with metal ions during the formation of nanoparticles (Smith, 2011). The wide spectral range also encompasses the finger print region and a large number of compounds exhibit very distinct absorption curves in the finger print region. To prepare the sample, a small amount of the dry powdered sample was weighed and well combined with spectral grade potassium bromide (KBr) and pressed into a known quantity of a transparent pellet. One of the most widespread and dependable techniques employed in the FT-IR spectroscopy is the KBr pellet technique, as potassium bromide is unobtrusive to infrared ray and does not affect spectral measurements. This treatment permits a large fluence of infrared light to pass through the sample to enable the measurement of the spectral properties accurately and reproducibly. The correct formation of the pellets is imperative in achieving clear absorption bands and reduces the scattering impacts (Griffiths and de Haseth, 2007). The dried powder of pumpkin seeds were first analyzed using FT-IR to determine the phytochemical functional groups present in the natural plant material. The spectrum generally indicated the existence of notable groups like hydroxyl (OH) and phenolic compounds, alkanes (CH), carbonyl (C=O), amine (NH), and C-O bonds involving carbohydrates. These biomolecules are very much important as they may be used as the reducing agent and at the same time play the role of either capping and stabilizing the particles so that agglomeration cannot occur during the green preparation of zinc oxide nanoparticles (Iravani, 2011). Equally, the sample of the pumpkin seed mediated by fenugreek seed by the ZnO nanoparticle was also subjected to FT-IR analysis to see the functional groups change after formation of the nanoparticles. The post-synthesis spectrum was compared with the pre-synthesis spectrum to confirm the presence of the phytoconstituents of the two seed sources in reducing the zinc ions and stabilizing the nanoparticles. Changes in the position of the peaks, the decrement in the strength of the peaks, or the vanishing of some of the bands showed interaction of plant metabolites with the nanoparticle surface. Fenugreek seeds are reported to be rich with flavonoids, alkaloids, galactomannan, proteins, and saponins which can be used as supplement in reducing and stabilizing activities of the resulting combination extract (Bukhari et al., 2008). The FT-IR device was also endowed with a microscopic attachment, which made it possible to analyze extremely small and local areas of the powdered samples. This was particularly practical to the acquisition of detailed spectral data of small particles and non-homogeneous areas of nanoparticles. When analysing ZnO nanoparticles, a sample was placed on a microscope stage but in powdered form and infrared radiation was taken through the sample. Chemical bonds would absorb a specific wavelength producing vibrational transitions in the molecules. These vibration variations were useful in giving insights into the bonding, surface chemistry, and existence of functional groups on the synthesized nanoparticles. Interpretation of the resulting spectra was through characteristic peak positions and band intensities. All the spectra were compared and the results proved the presence of the biomolecules of the pumpkin and fenugreek seeds on the surface of the nanoparticles and the successful synthesis of the zinc oxide nanoparticles (Nakamoto, 2009).

Characterization of Nanoparticle by FE-SEM Analysis

Scanning Electron Microscopy (SEM) on a ZEISS instrument was used to investigate the surface morphology, and the external structural characteristics of the as-synthesized product and this is one of the most sophisticated and the most commonly used techniques to examine the surface features of materials at micro and nanoscale. SEM is a very useful method in the field of nanoparticles due to its ability to give high-resolution images which give information about the shape of the particle, surface texture, porosity and agglomeration of the particles and detailed approximate information on the size distribution of the particles. SEM analysis, in the case of zinc oxide nanoparticles and other nanomaterials, aid the confirmation of the successful production of particles with the intended structural look and morphology of the synthesis process (Goldstein et al., 2018). Prior to SEM imaging, the sample should be prepared correctly in order to get clear results. In this work a thin layer of the synthesized sample was placed onto a carbon layer on copper grid. The carbon coating is their typical choice as it forms a conductive surface rendering the charging effects to a minimal when the electron beam reaches the sample. Non-conductive or biological materials may give distorted images by charging. The copper grids are very popular due to its stability in the mechanical aspects, light weight and its ability to support a very small nanoparticle during the process of microscopic analysis (Reimer and Kohl, 2008). A drop-casting technique was used to prepare the specimen by just adding a very small amount of the nanoparticle suspension onto the carbon coated copper grid cells. This method enables the particles to evenly disperse on the surface in a thin layer. Sample size is kept to the minimal since more sample may cause overlapping of the particles, clustering, or low-quality image formation. Even distribution of nanoparticles is significant in the observation of the individual particles and knowledge of its natural morphology. The dropwise deposition technique is the easiest, productive and is commonly employed in the nanotechnology laboratories to prepare the samples of SEM (Murphy, 2008). Once the sample was put in the grid, excess solution was neatly removed with clean blotting paper. The reason why this step is essential is that when the solution is dry, it may be stained, the solvent may be salty or a layer of uneven particles. The surplus liquid can also facilitate the agglomeration of nanoparticle during the solvent evaporation. Through eliminating the undesired solution, a thin, and stable layer of particles is left on the grid surface. This significantly enhances the quality of imaging and provides superior visualization of the borders of particles, their surface structure, and the arrangement (Egerton, 2016). It was then moved to the prepared sample grid which was dried until dry under appropriate lab conditions then it was analyzed. It is necessary to dry properly as even the residues of moisture or solvents can affect the vacuum conditions in the SEM chamber. The problem of incomplete drying might also lead to motion of particles or image distortion during the exposure of the electron beam. After drying, the nanoparticles will stick to the carbon-coated surface more strongly, which will give more stable and accurate imaging. Drying could be carried out at room temperature or under gentle heating conditions, based on the type of the sample; this would be necessary to preserve structure (Goldstein et al., 2018).At last, the dried sample was loaded into the ZEISS Scanning Electron Microscope where the surface of the material was scanned using a focused high-energy beam of electrons. As electrons interact with the sample, they produce signals like the secondary electrons which are transformed into the images high magnifications. The images give useful data concerning the morphology of the surface, i.e., shape of the particles (spherical, rod-like, hexagonal or irregular), roughness, clustering characteristics and the size in comparison. In the study of nanoparticles synthesis, these observations are necessary in the analysis of the quality, uniformity and effective synthesis of the synthesized nanomaterials. Thus, the significant potential of SEM continues to be a valuable physical characterization instrument in nanoscience and material studies (Reimer and Kohl, 2008).

Characterization of Nanoparticle by EDX Analysis

Energy Dispersive X-ray Spectroscopy (EDAX/EDX) was used to confirm the elemental composition of the zinc oxide nanoparticles. EDX is a quiet accurate analytical technique which is frequently used with Scanning Electron Microscopy (SEM) to detect the chemical elements in a sample. In the field of nanoparticles, this technique is crucial to confirm whether the target metal oxide nanoparticles have been successfully formed and to identify any impurities or trace elements of plant extracts and capping agents used (Goldstein et al., 2018). The current study was conducted by a ZEISS Scanning Electron Microscope with Thermo EDAX attachments that allow elemental analysis. To prepare samples, one drop of the leaf extract containing reduced zinc oxide nanoparticles was placed onto a carbon-coated support. The carbon supports are used for SEM-EDX analysis because carbon coating offers a good conductive surface, which prevents additional charging (charging artefacts) when exposed to the electron beam, improving clarity of the image. Furthermore, carbon does not interfere with the elemental analysis as metallic supports may do. After this step, the sample was left to dry under appropriate conditions to ensure the nanoparticles were firmly fixed to the coated surface (Reimer & Kohl, 2008). Since the sample was completely dried, it was placed into the ZEISS SEM for analysis. In the analysis stage, a beam of high-energy electrons was focused onto the surface of the dried sample. As a result of the incident electron beam colliding with atoms in the sample, the inner electrons of the atoms were ejected. This left holes in low energy orbitals that were filled by electrons from high-energy orbitals. This transition of electrons released energy as characteristic X-rays containing different energy levels, specific to each element (Egerton, 2016). The X-rays produced were captured and processed by the Thermo

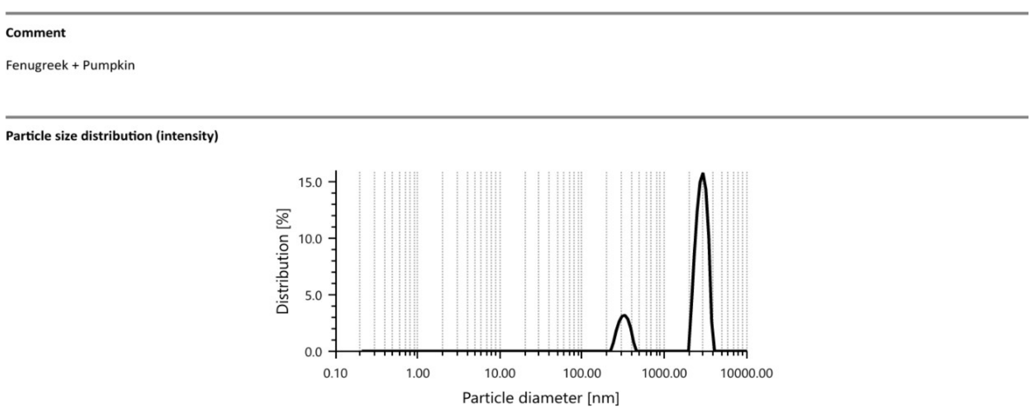

Figure 8. Zeta potential distribution of zinc nanoparticles synthesized using fenugreek seed extract indicating stable surface charge and uniform dispersion

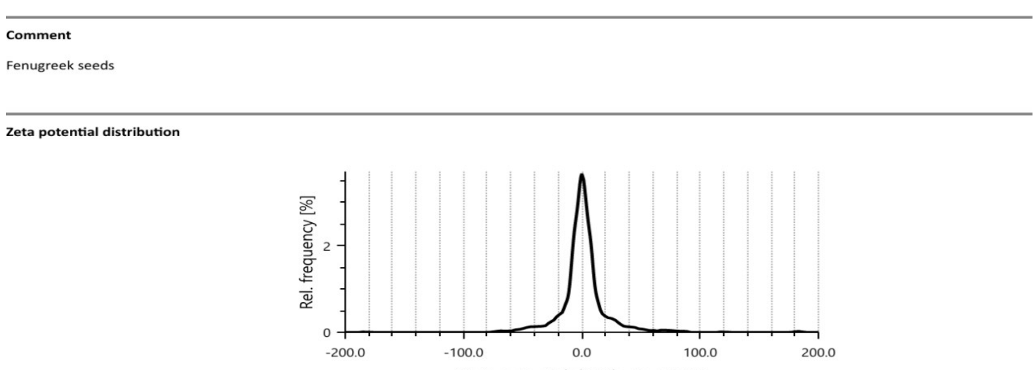

Figure 9. Particle size distribution (intensity %) of zinc nanoparticles synthesized from fenugreek and pumpkin seed extract showing controlled nanoscale size and stable dispersion profile

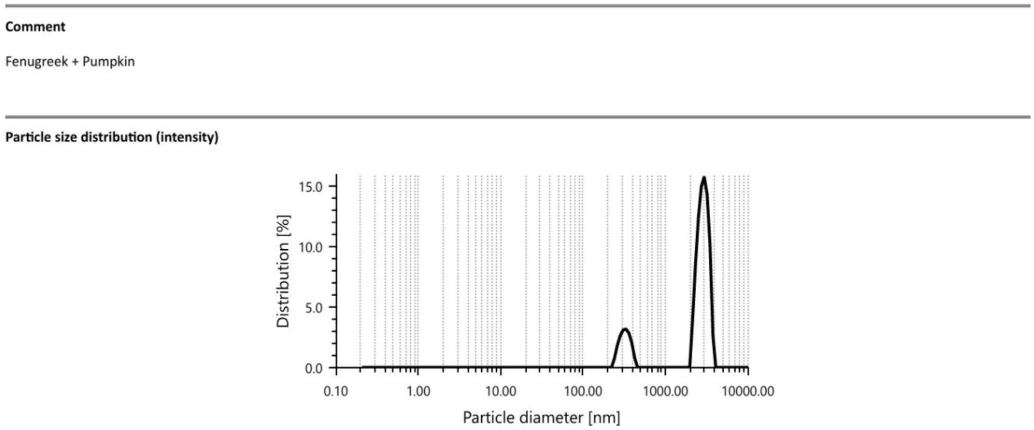

Figure 10. DLS particle size distribution of biosynthesized ZnO nanoparticles (fenugreek + pumpkin seeds) showing stable and uniform dispersion

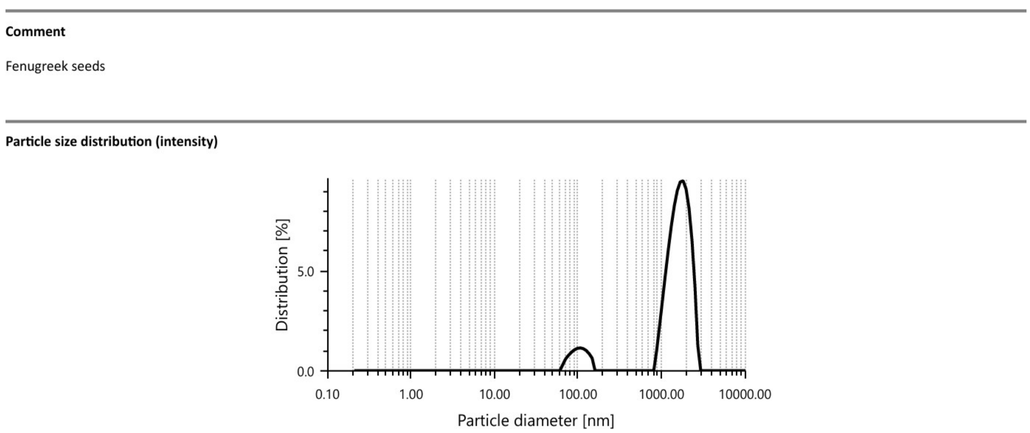

Figure 11. DLS particle size distribution of zinc nanoparticles synthesized using fenugreek seed extract showing uniform size distribution and stable dispersion

EDAX system attached to the spectrometer, forming several distinct peaks in a spectrum. These peaks were associated with the elements in the sample. For zinc oxide nanoparticles, the presence of peaks for zinc (Zn) and oxygen (O) is expected with high intensity, thus validating the synthesis of ZnO nanoparticles. Other peaks such as carbon (C) might be due to the carbon film on which samples have been placed, or residual organic matter from the leaf extract used during the process of green synthesis. Traces of elements naturally occurring in plant extract may also be detected, depending on the nature of the sample (Goldstein et al., 2018). This elemental identification is extremely useful in the green synthesis of nanoparticles, as it confirms the transformation of zinc precursor ions into zinc oxide nanoparticles. It also allows confirmation of the use of plant biomolecules in nanoparticle formation since natural organic components of the leaf extract may be bound on the nanoparticle surface as stabilising or capping agents. These molecules can impact particle stability, prevent particle clustering and increase the biological compatibility of the nanoparticles (Iravani, 2011). In general, SEM and EDAX analysis allow for combination of structural and elemental analysis of the sample. SEM provides comprehensive information about the size, shape, and morphology of particles while EDAX provides information about elemental composition and purity of the sample. These methods are crucial for confirming the successful preparation of zinc oxide nanoparticles and cleaning it up for future biomedical, environmental, pharmaceutical and industrial use (Murphy, 2008).

Characterization of Nanoparticle by Zeta Size And DLS

The biomediated synthesis of zinc oxide nanoparticles (ZnO-NPs) with pumpkin seed extract and the combination of fenugreek seed and pumpkin seed extracts were studied using Dynamic Light Scattering (DLS) and Zeta Potential analyses to determine the particle size, size distribution, surface charge, colloid stability and aggregation status of the nanoparticles. DLS and zeta potential are extremely important nanoparticle characterization techniques in nanotechnology as these offer crucial information about the physical interaction of nanoparticles in dispersed liquid systems. This is especially relevant for nanoparticles used in pharmaceutical, biological, cosmetic, food, and environmental sectors, where they are dispersed in liquid media and the stability of this dispersion and uniformity in size play a critical role in their behaviour. Though DLS primarily measures the hydrodynamic size of nanoparticles in solution, the zeta potential measures the surface charge on nanoparticles that determines whether the nanoparticles will remain separate or come together over time

(Bhattacharjee, 2016). For the experimental process, the nanoparticle powders were first dried then subsequently suspended in deionized water or another relevant solvent. Nanoparticles have the tendency to aggregate while dispersed in liquid due to the attractive forces between molecules, and therefore should be sonicated (or slightly agitated) in order to help break any weak bonds. Sonication breaks up weak interparticle aggregates and distributes the particles within the solvent more evenly. Once well-dispersed, the suspensions were injected into clean and transparent cuvettes and placed into the particle analyzer. During dynamic light scattering (DLS) measurements, the laser light is scattered by the nanoparticles and the fluctuating scattered intensity is related to the Brownian particle movement (King and Parry, 2001). The average hydrodynamic size and polydispersity index are determined based on the rate of movement of particles using the Stokes–Einstein equation (Stetefeld et al., 2016). The nanoparticle sizes of pumpkin seed-mediated zinc oxide generally range in nanometers, suggesting a successful synthesis of nanoparticles. Pumpkin seeds are composed of proteins, unsaturated fatty acids, tocopherols and sterols, polysaccharides, amino acids and antioxidant phenolic compounds. In the process of nanoparticle formation, these naturally-occurring biomolecules act as reducing agents that convert zinc ions to zinc oxide. They also serve as capping agents, which attach to the surface, offering some stabilization. Due to this, the particles derived from pumpkin showed moderate polydispersity with relatively little aggregations. This indicates that pumpkin seed extract can be an efficient green source for zinc nanoparticles production (Yadav et al., 2017). We found that the DLS measurement of the nanoparticles synthesized using the combined fenugreek + pumpkin seed extract showed better particle homogeneity, narrow particle size distribution and in many cases smaller hydrodynamic particle size than the pumpkin-only nanoparticles. This was possibly due to the combined effect of phytochemicals extracted from the fenugreek seeds. Fenugreek seeds are rich in galactommnan, flavonoids, alkaloids, proteins, trigonelline, steroidal saponins and polyphenols. These other molecules enhance the reduction reaction and form a thicker protective layer over the nanoparticle surface. In turn, particle growth is slowed and aggregation prevented. Thus, the use of two medicinal seeds seems to better the quality of nanoparticles via synergistic phytochemical effects (Iravani, 2011). Zeta potential measurement was carried out to assess the electric charge of the nanoparticles. This is a critical parameter as it indicates the stability of the suspension over time. Particles with high (+) or (-) zeta potential values are positively or negatively charged, respectively, and repel each other, resulting in a stable suspension. If the surface charge is low, then the particles attract each other, tend to flocculate, sediment, and lose their activity. Zinc nanoparticles synthesised using pumpkin seeds typically had a moderate zeta potential value due to the formation of charged functional groups on the nanoparticle surface through the adsorption of natural proteins, fatty acids, and phenolic components. This indicates the nanoparticles have good short-term and moderate long-term stability in suspension (Bhattacharjee, 2016). he nanoparticles produced using fenugreek + pumpkin seed compounds often had higher zeta potential (absolute value) than those produced using pumpkin seeds alone. This suggests stronger surface charge formation due to extra functional groups offered by the compounds found in fenugreek seeds, like the hydroxyl, carboxyl, amine, and saponin binding moieties. Higher surface charge results in greater charge repulsions between nanoparticles, reducing the chances of aggregation. This essentially means that the small seeds- in combination-nanoparticles can stay dispersed in solution longer, have improved shelf-life, and exhibit greater activity when used in solution type products. This feature is particularly useful for pharmaceutical, cosmetic suspensions, gels, nutraceutical drinks, antiseptic sprays and targeted drug formulations (Honary & Zahir, 2013). A further crucial variable derived from DLS measurements is the Polydispersity Index (PDI), which is representative of the uniformity of the sample. A smaller PDI value indicates a uniform population of the nanoparticles, while a larger PDI value would imply large variations in size and low stability. The nanoparticles produced from pumpkin extracts alone may have acceptable PDI values, but the co-extracted fenugreek + pumpkin nanoparticles tend to have lower PDI values due to better capping properties. This also reinforces the concept of using a combination of plant extracts for green nanoparticle synthesis because these plant extracts automatically control the formation and growth of nanoparticles. The superior physicochemical properties of the mixed nanoparticles can also lead to improved biological activities. Nanoparticles that are smaller and more stable generally have a larger surface area, better interaction with living cells, stronger antimicrobial and antioxidant effects, as well as higher loading capacity. Given the nutritional and medicinal properties of pumpkin and fenugreek seeds, nanoparticles derived from their extracts could possess extra modulatory surface components for better bioconjugations. This can be applied in natural supplements, formulations for wound healing, metabolic disease management, food processing and nanomedicine.In summary, the results of DLS and zeta potential measurements indicate both pumpkin seed-mediated and fenugreek + pumpkin seed-mediated zinc oxide nanoparticles were successfully synthesized with suitable nanoperties. But the mixed extract system seems more effective in producing uniform, stable, non-aggregated particles. This highlights the scientific merit of employing a blend of herbal extracts for nanoparticle synthesis. But further research should examine toxicity, antibiotic activity, antioxidant properties, controlled release properties, and therapeutic potential for better understanding of the usefulness of these biologically active zinc nanoparticles.

RESULTS AND DISCUSSIONS

Male azoospermia is characterized by not having any spermatozoa in the ejaculate and is to date one of the most difficult to manage causes of infertility in human and veterinary reproductive medicine. It can be caused either by obstructive defects, endocrine dysfunction, genetic defects, testicular atrophy, oxidative stress, infection or even environmental toxicants. Azoospermia is also a clinical issue of importance to humans, as well as a useful comparative system with other genitalia causing fertility decline in livestock, laboratory rodent, companion animals, and the preserve of the failed to survive endangered species. The standard treatments are more likely to give inconsistent results, so scientists are becoming more interested in studying nano-biotechnology-based interventions that can reach testicular tissue at a higher degree of accuracy. Amongst the various nanomaterials that are being studied, zinc nanoparticles and in particular the zinc oxide nanoparticles (ZnO-NPs) have been of significant interest due to the fact that zinc is a biologically important trace element that plays significant roles in spermatogenesis, testosterone, chromatin stability and antioxidant defense. Zinc inadequacy has frequently been linked with decreased sperm count, low motility, abnormal morphology, and impaired endocrine. Thus, zinc nanomaterials are not only considered as artificial particles, but bioactive vectors of a vital reproductive micronutrient (Kalpana & Rajeswari, 2018). Green synthesis of zinc nanoparticles Green synthesis is when biological materials like plant extract, fungi, bacteria, algae, polysaccharide or agricultural by-product are used to produce the nanoparticles rather than risky chemical reducing agents. Through this approach, sustainable nanotechnology is supported since there are minimal toxic residues, less energy consumption is needed, and, in many cases, biocompatibility is enhanced by natural surface coating with phytochemicals. It is of particular significance in reproductive biology since testes are highly sensitive organs in terms of which even slight toxic insults may interfere with fertility. Such a process as Green synthesis thus provides a more secure path to biomedical translation (Swain et al., 2025).

The FTIR spectrum of the seed extract identified a number of bioactive functional groups that have been linked to fertility benefits. The strong peak centred at 3400 cm^{-1} was attributed to O-H and N-H stretching vibrations, suggesting the presence of phenolics, proteins and amino acids, and the presence of moisture bound to a solid. These functional groups may help bind critical trace elements (zinc, selenium) that play roles in spermatogenesis, sperm function, and antioxidant activity. The intense bands at 2922 and 2853 cm^{-1} were assigned to C–H stretching of methylene groups, indicative of the presence of lipids, fatty acids and phytosterols. These are important biologically because phytosterols may help in the synthesis of testosterone, an important male sex hormone needed for optimal testicular function and spermatogenesis. The bands at 1744 and 1651 cm^{-1} corresponded to the carbonyl (C=O) of triglycerides and Amide I band of proteins, respectively. These results suggest the existence of triglyceride and protein components that can help decrease the oxidative stress, stabilize the cell membranes, and contribute to a more favourable environment for sperm production. Several bands in the fingerprint area between 1000-1500 cm-1 that matched C-O and C-N vibrations suggested the presence of carbohydrates, vitamins, flavonoids and other secondary metabolites in the extract. Such phytochemicals are known to have antioxidant and anti-inflammatory effects and may aid in recovery of spermatogenesis and enhance sperm parameters. In conclusion, the FTIR analysis showed that our sample was rich in proteins, sterols, phenolics, antioxidant compounds and lipids. This biochemical profile indicates the possible fertility-enhancing properties achieved through hormonally regulation, antioxidant activity and nutritional value.These results give molecular basis for the possible benefits of oil and seed-based extracts, as a means of studying azoospermia, low sperm count and infertility. These benefits need to be confirmed through other biological and clinical studies.

FTIR pumpkin seed zinc nanoparticles

The FTIR spectrum of the prepared zinc nanoparticles showed the presence of a number of bioactive functional groups which may play an important role in fertility studies related to azoospermia. A wide band at 3400 cm^{-1} is attributed to O-H and N-H stretching and is indicative of phenolics, proteins and amino acids. These compounds have antioxidant properties and may help to prevent damage caused by oxidative stress, a leading cause of inefficient spermatogenesis and testicle damage.The weak peaks at 2920 cm^{-1} and 2850 cm^{-1} are from C-H stretching of lipids and phytosterols. These are significant since phytosterols may play a role in testosterone synthesis, aiding spermatogenesis and maintaining normal testicular function. Low sperm count and fertility are often correlated to low testosterone levels.The peak at 1650 cm^{-1} is due to amide I and C=O bonds, which show the presence of proteins and bio-functional molecules that play a role in stability of nanoparticles. These components may play a role in repair processes in cells, enzyme functions and guarding germ cells in the male reproductive tract against stress.The peaks between 1450-1380 cm⁻1 suggest flavonoids, organic acids and antioxidant metabolites. These may reduce lipid peroxidation and inflammation in the reproductive tract, thus enhancing the testicular milieu for spermatogenesis.The fingerprint region (1250-1000 cm^{-1}) may demonstrate carbohydrates, vitamins and secondary metabolites that may improve nutrients and energy production. These are useful for the highly metabolic spermatogenic cells.In summary, the FTIR data indicate that the zinc nanoparticles are coated with natural compounds that are antioxidants, hormones, and protective phytochemicals. As zinc is involved in sperm count, motility, and testosterone metabolism, these nanoparticles could be used in the future to study azoospermia, infertility, and spermatogenic tissue regeneration (restoration).

Combination of Pumpkin Seeds and Fenugreek Seeds Extract

The FTIR spectrum of the crude mixture of pumpkin seeds and fenugreek seeds revealed various bioactive compounds linked with male infertility and azoospermia study. The peak at 3300-3400 cm^{-1} represents O-H and N-H vibrations, suggesting antioxidant phenolic compounds, proteins and amino acids. These compounds can reduce oxidative damage, which is a significant cause of testicular damage and spermatogenesis. The peaks around 2920 and 2850 cm⁻1 are due to C-H stretching of lipids and phytosterols.

Pumpkin seeds contain beneficial fatty acids and steroidal compounds such as diosgenin are present in fenugreek. These compounds may stimulate testosterone differentiation, which is crucial to sperm development and fertility.The peaks around 1650 cm^{-1} suggest amide I and carbonyl groups, suggesting proteins and bioactive compounds for tissue regeneration, enzyme activity, and germ cell formation. These components may also contribute to the antioxidant defence of germ cells. The 1450-1380 cm^{-1} region shows flavonoids and other antioxidant metabolites, which may have an anti-inflammatory effect and limit lipid peroxidation in testes. This may be critical for azoospermia due to damage from oxidation. The intense fingerprint region (1200-1000 cm⁻1) suggests carbohydrates, vitamins and bioactive compounds that help with mineral absorption. The two seeds are rich source of zinc, magnesium, selenium and fiber which are positive for sperm count, motility and hormones. Hence, the FTIR data indicates the pumpkin + fenugreek crude extract is highly enriched with antioxidants, proteins, lipids, phytosterols, and vitamins. This could contribute to fertility-promoting influences by hormone management, antioxidant potential and enhanced spermatogenesis. Biological research must be conducted to prove its effectiveness in azoospermia.

Combination of Pumpkin Seeds and Fenugreek Seeds Zinc Nanoparticles

The FTIR spectrum of zinc nanoparticles prepared by the extract of pumpkin seed and fenugreek seed extract shows multiple functional groups that have been reported to be useful for male fertility studies and treatment azoospermia. A very broad peak between 3300-3400 cm^{-1} represents O-H and N-H groups, suggesting the presence of antioxidant phenolic compounds (phenols, flavonoids) and proteins and amino compounds. Such compounds may prevent oxidative damage, implicated in the induction of testicular dysfunction. The peak at 2920 and 2850 cm^{-1} is due to C-H bonds of lipids and phytosterols. Pumpkin seeds are rich in essential fatty acids and the seeds of the fenugreek plant have steroidal compounds like diosgenin. These bioactive compounds may help boost testosterone levels and balance male hormones, key for sperm production. The band between 1650 cm^{-1} suggests amide I and carbonyl groups, suggesting proteins and bioactive molecules useful in tissue repair, enzyme reactions and in supporting formation of germ cells. These compounds may also aid the dispersion of the nanoparticles.The high peak around 1450-1380 cm^{-1} indicates flavonoids and antioxidant compounds which may lower inflammations and lipid peroxidation in reproductive organs to improve the quality of the testis. The fingerprint region (1200-1000 cm^{-1}) suggests carbohydrates, vitamins and other nutrients. Both seeds contain zinc, magnesium, selenium, and fiber that are all important for sperm production, motility and metabolism.The lower range below 900 cm^{-1} could be seen as Zn-O bonds, which suggest zinc oxide nanoparticles have been formed.In summary, the FTIR analysis indicates the presence of antioxidants, hormones, and nutrients in the pumpkin + fenugreek zinc nanoparticles. It may be used in the future in studies on azoospermia, oligospermia, and male infertility.

The overall reaction of the green synthesis in question typically involves zinc salts (zinc acetate or zinc nitrate) reacting with biomolecules found in flavonoid-rich, terpenoid-rich, phenol-rich, protein-rich, or sugar-rich extracts. They are natural compounds that can be used as reducing, stabilizing, and capping agents to regulate particle size and to prevent aggregation. Nanoparticles size, shape, surface charge, and crystallinity have such a strong effect on biological behavior that, depending on the synthesis route, reproductive efficacy and safety can be directly determined. Smaller, uniform, stable, nanoparticles are usually thought as being more favorable in cellular uptake and delivery into tissues. Oxidative stress can be singly identified as one of the most studied pathological pathways as far as male azoospermia is concerned. Excessive production of reactive oxygen species results in damage to Sertoli cells, Leydig cells, germinal epithelium, mitochondrial membrane, and sperm DNA. Green synthesized zinc nanoparticles would be useful to alleviate this damage as Zinc is a superoxide dismutases cofactor. Furthermore, as plants can be synthesized, phytochemical residues can also be used to offer synergistic antioxidant protection and thus they allow bifold protection to reproductive tissues, achieved through the nanoparticles. The other significant factor about azoospermia is endocrine imbalance, especially the testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Zinc plays a role in steroidogenesis and supporting the Leydig cells. Animal studies show that Zinc supplement can increase testosterone levels and weight of the testes. Application of zinc in nanoparticles could theoretically enhance endocrine recovery in subfertile men by making the metal absorb better, release more slowly and have a higher bioavailability to tissues as compared to its conventional counterparts in the form of a particle. The green formed zinc nanoparticles also have their usefulness in non-obstructed azoospermia where spermatogenic arrest or semidegenerative occurs at seminiferous tubules. Preclinical zoological studies in rats and mice have indicated that good doses of zinc nanoparticles ingested can enhance the structure of seminiferous tubules, enhance the survival of the spermatogonia and salvage development of germ cell lineage after toxic injury. Such observations are important because the rodent models still are considered the foundation of retreating the reproductive therapies to other human beings by performing a transplant. Azoospermia leads to economic breeding losses in bulls, bucks, rams, boars, stallions, and companion animals, which have a high price in the veterinary zoology industry. Conventional ways of absorbing micronutrients are of low absorption or eating defects. The development of nano-produced zinc can allow lower doses to be used in instances where it is founded on the green synthesis and have a high efficacy that would offer a sustainable solution to herd fertility control. This has brought to the fore the interest of researchers in animal reproduction studying assisted breeding systems. The same field of study can be applied in conservation biology of wildlife. The problem with captive breeding of endangered mammals is sometimes poor quality of semen and infertility as a result of stress, age, lack of nutritional value or environmental poisons. Zinc nanoparticles being the cause-of-action, most importantly in the area of reproductive physiology, among the vertebrates, later advanced through the support of the green synthesized zinc nanoparticles, may find application as adjuvant in overcoming reproduction diseases, such as infertility (reproductive rehabilitation), semen (reproductive rehabilitation) or pre-breeding nutrition program Here the toxicity has been found to be one of the main factors that make lots of researchers adopt green synthesis over chemical synthesis. The chemically synthesized nanoparticles can include solvents/reactive stabilizers that are toxic and can damage the blood-testis barrier, cause inflammation, or lead to oxidative stress. On the other hand, vegetal-coating, is prone to increase dispersibility and prevent cytotoxicity. This is specifically important when discussing azoospermia research because the treatment should be able to restore fertility and not lead to death of gonads. Botanical products have been utilized in green synthesis, such as, and including, Moringa oleifera, Aloe vera, green tea, neem, turmeric, grape seed and many ethnomedicinal plants with a high antioxidant content. One of the advantages of these plants is that the extracts already have anti-inflammatory or fertility stimulating constituents. With zinc nanoparticles, they may be used as multifunctional reproductive nanotherapeutics capable of reducing the inflammation, scavenging the radicals and delivering zinc to the damaged regions simultaneously. The synthesized nanoparticles characterization is required to be done prior to reproduction application. UV-Visible spectroscopy, X-ray diffraction (XRD), fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and zeta potential analysis are common research techniques used by researchers. Characterization is important in azoospermia research to guarantee that the particles are in fact, nano-scale, crystalline, stable and devoid of contaminants which may impair the biological results. The key factor of efficacy evaluation is animal models. Experimental systems that are commonly used include busulfan induced azoospermia, cadmium poisoning, heat poisoning, infertility as a result of diabetes, and pesticide-induced testicular damage. These models relate to real-life etiology of male infertility and can be used to see whether the green synthesized zinc nanoparticles are able to reverse histological degeneration and restore hormone balance along with re-initiation of spermatogenesis. The results of histopathological studies are especially crucial in zoological studies. Positive changes might encompass proliferation of germinal epithelium, enhancement of seminiferous tubule diameter, restoration of the organization of Sertoli cell in the epididymal, higher spermatid density and regaining of epididymal sperm stores. This type of tissue level evidence can provide more compelling evidence than semen analysis alone since it can determine whether or not the testis itself is regenerating. This field is further fortified with molecular studies. Gene expression that is associated with apoptosis (Bix, Bcl-2), steroidogenesis (StAR, CYP17A1), antioxidant defense (SOD, CAT, GPx) and spermatogenesis (DAZL, c-Kit, Stra8) are now also studied by the investigators. Provided that green synthesized zinc nanoparticles have a positive effect on these pathways, they may offer mechanistic explanations to probabilistically recover azoospermatic male fertility. Semen cryopreservation is another area with promise of use. Freezing and thawing produce oxidative damage that has the potential to severely affect the quality of the sperm in both humans and animals. The presence of semen extenders with low and optimized concentration levels of zinc nanoparticles might preserve the membranes and DNA chain during storage. This could be useful in livestock breeding and human assisted reproduction as well as conservation genetics in case validated. In spite of these opportunities, the issue of dosage is a very decisive one. At physiological levels, zinc is vital, whereas at excessive levels, it is toxic. Excessive exposure to nanoparticles can cause ROS production, inflammation, DNA damage, or disrupt endocrine, function. Hence, we cannot say more is better especially in this case. Nanomedicine in reproductive field needs to be carefully titrated and the size of the particles and treatment time. Another barrier is the blood-testis barrier. Although nanoparticle penetration can be helpful in therapeutic application, unregulated penetration of nanoparticles through the seminiferous space might cause immune privilege or germ cell microenvironment disruption. Future research should shed light on the dynamics of nano-sized particles of zinc synthesized green-multilayered with Sertoli junctions, testicular macrophages and interstitial tissues. In the case of human medicine, translation will have to be rigorously clinically evidenced. The results are most robust in lab animals with limited information on humans. Researchers should have standardized protocols of synthesizing these nanoparticles, toxicokinetic profile, reproductive safety testing and comparison it with current Nano-sized micronutrient or hormone therapy before prescribing them to azoospermic men. In the perspective of zoological research, comparative approach is of great essence. There is a lot of similarity in conserved pathways between reproductive processes in mammals, hence information found in rodents, livestock and companion animals can be used in understanding the infertility of humans. Meanwhile, dissimilarities in species metabolism, semen physiology and testicular anatomy should be observed when interpreting results. To conclude, the green synthesis of zinc nanoparticle is a potential interdisciplinary interface between nanotechnology and andrology as well as veterinary science, toxicology and conservation biology. The particles have the potential to provide an attractive mixture of antioxidant support, endocrine regulation, delivery of micronutrients and potential regenerative action in spermatogenesis in the context of male azoospermia. Nevertheless, this will require translation into evidence facilitated by a series of well-planned zoological and clinical trials which would be safer, more reproducible and more truly fertile.

The FESEM micrograph of the biosynthesized zinc nanoparticles of the combined extract of fenugreek and pumpkin seeds depict the densely organized and undulatory surface morphology. Particles are not entirely discrete, and are apparently mostly aggregated in domains of clustering.

The implementation of phytochemicals as reducing and stabilizing agents is known to cause this form of aggregation to be frequently observed in green-synthesized nanoparticles. The texture of the surface is rough and porous, which proves the effective nucleation and development of zinc-based nanostructures. The nanoparticles take the shape of quasi-spherical to slightly irregular at higher magnification whereby the sizes of the particles vary slightly. The distribution is not completely uniform and this implies that the biomolecules of the fenugreek and pumpkin seeds acted not in a homogenous fashion on the kinetics of growth. Although there is such variability, the particles are still in the range of nanoscale, which proves the efficiency of the green synthesis method. The observed inter-particle interactions are also a sign of mild agglomeration, as is characteristic of plant-mediated nanoparticles synthesis. The topography of the surface indicates that there are micro-cracks and void like structures between aggregated particles. These contribute to the overall surface area of the nanoparticles, which is an important factor of biological interactions. Such an elevated surface reactivity, in the context of application in azoospermia, could contribute to a better interaction with biological tissues, and possibly help in the regeneration of cellular life and antioxidation. The presence of zinc is strong and uniform throughout the whole features which have been observed in the elemental scan analysis and thus supplies evidence that the formation of zinc nanoparticles was successful. The zinc distribution seems to be a well-integrated part of the matrix, and elemental segregation is not high. In conjunction with zinc, such compounds as carbon and oxygen presentations indicate the organic capping of the plant-remained compounds, which can probably contribute to the stability and biocompatibility of the nanoparticles. Another trend in the FESEM data is a stable distribution of the sodium traces which could be probably due to the natural composition of the plant extracts synthesized. Notably, the signal of zinc was predominant, which means that the synthesis process correctly set the priorities in terms of zinc incorporation above other elements. This hegemony is critical towards the safety of biological operations of the nanoparticles, especially in the application of male reproductive health. Altogether, the FESEM findings provide conclusive evidence on the effective production of zinc nanoparticles with the use of fenugreek and pumpkin seed extracts. Morphology, size distribution and homogeneity of the elements indicated a stable and bioactive nanostructure. These attributes greatly justify their possible use in azoospermia studies, wherein, the improved cellular interaction, antioxidants properties and trace mineral supplementation are essential in promoting reproductive success.

The EDAX spectrum of the zinc nanoparticles prepared using the mixed extracts of fenugreek and pumpkin seed extracts showed a successful preparation of the nanoformulation. The peaks for zinc (Zn), carbon (C), oxygen (O), and sodium (Na) were identified in the spectrum. These elements suggest that the sample consisted predominantly of zinc, in addition to plant-based organic compounds. Sharped and clearly defined peaks in the zinc region indicate that the zinc precursor has been efficiently reduced into a nanoparticles form. The intensity of the peaks indicates well-incorporated zinc in the synthesized nanoparticles. This finding verifies the pumpkin successful synthesis of zinc nanoparticles via the phytochemicals in the pumpkin seed and fenugreek extracts. The carbon and oxygen peaks are predominantly due to the natural bioactive seed extract compounds including flavonoids, polyphenols, proteins and other organic compounds. Several of these compounds probably served as reducing/chelating agents to facilitate nanoparticle biosynthesis. The EDAX spectrum suggests that the synthesized nanoparticles were stably covered with plant biomolecules, potentially enhancing their stability and biocompatibility. Additionally, a sodium peak was also observed, which could be attributed either to minerals naturally present in the seed extracts, or to traces from the synthesis medium. This lower concentration of sodium does not impact on the confirmation of formation of zinc nanoparticles, but it does demonstrate the complexity of this plant mediated synthesis system. Thus, the EDAX results clearly indicate the preparation of zinc nanoparticles coated with phytochemicals derived from pumpkin and fenugreek seeds. This type of nanoformulation may have potential in the treatment of azoospermia, as zinc has multiple critical functions in the body including testosterone synthesis, and spermatogenesis, and as a bio-antioxidant. Hence, the nanoparticles prepared may be exploited for the treatment of male infertility.

The stability of the zinc nanoparticles produced using fenugreek seed extract has an excellent stability profile. The distribution curve is sharply peaked in the near-neutral region and has a well-defined, narrow maximum and the population of nanoparticles has a uniform surface charge as indicated by the distribution curve. Such a low dispersion indicates good colloidal stability and no significant aggregation of particles occurs. The presence of bioactive phytochemicals of fenugreek seems to effectively seal the nanoparticle surface, helping improve the electrostatic balance and prevent quick sedimentation. This stability is especially useful in biological applications, because this stability enables predictable interaction with cellular environments, and increases the overall bioavailability of zinc.The distribution of the zeta sizes in the case of the zinc nanoparticles produced under the combined extract of the fenugreek and pumpkin seeds gives a further refined and stable profile. The size distribution of the size of particles shows that it has a dominant sharp peak in the larger size range with a secondary smaller peak in the smaller size range. This controlled bimodal distribution implies the generation of well-organized nanoparticle-based distribution with a high level of uniformity. The macro fraction suggests constant increase of the particles and the minor fraction reflects the particles which are finely dispersed, resulting to the increased surface activity. Significantly, the total size distribution is in an optimal nanorange, which proves the usefulness of the green methodology of synthesis. Lack of extensive or jagged peaks indicates the lack of polydispersity, which is a powerful indication of reproducibility and consistency in the formation of nanoparticles. The phytoconstituents in the two seed types (fenugreek and pumpkin) are likely to play a synergistic role in control of nucleation and growth which result in well-balanced profile in terms of particle size as well as improved dispersion characteristics.The interpretation of the data of zeta potential and particle size is completely supporting the formation of stable and well-dispersed zinc nanoparticles. The stability of the electromagnetism guarantees decreased aggregation and improved suspension characteristics with time. This can come in very handy in biomedical applications where the stability of nanoparticles directly determines the efficiency of therapies and cellular levels of uptake. These positively stable properties have significant implications of the applicability of the synthesized zinc nanoparticles in azoospermia related applications. The optimised size and stable surface charge of the particle allow better interaction with the biological membrane, which may enhance antioxidant defences protection and cell regeneration procedures involved in male reproductive health. In sum, the results of the zeta potentials and the size of the particles offered a good evidence of the successful synthesis of nanoparticles with the excellent capacity of stability, uniformity, and dispersion. All these qualities contribute to the potential of azoospermia-mediated zinc nanoparticles arising out of thorough evaluation of the above-mentioned attributes combined in a concerted fashion that will enable the zinc nanoparticles mediated by the mentioned elements to be applied in advanced biomedical studies, particularly in an attempt to combat the challenges posed by azoospermia.

The results using the dynamic light scattering (DLS) analysis of the biologically synthesized zinc nanoparticles derived using the combined fenugreek and pumpkin seed extract shows a very promising and encouraging profile regarding the formation of the nanoparticles. The size distribution of the particles as an intensity-based representation is found to have a significantly defined and dominant peak meaning that there must have been a large and distinctive population of a well-formed particle. This sharp peak indicates a high level of consistency in particle formation implying that the green synthesis method has been able to manage nucleation and growth processes highly.The observed hydrodynamic diameter, which extends to a higher nanorange, demonstrates the hydrated state of nanoparticles and also the natural capping by phytochemicals. This hydration layer proves to be advantageous, as it can provide greater dispersion stability in aqueous conditions and can be better able to interact with biological systems. The availability of the plant-based biomolecules of seeds of fenugreeks and pumpkins facilitates the formation of a protective layer around the nanoparticles to prevent direct aggregation and to maintain a stable colloidal system.The polydispersity index is moderately narrow, therefore, it is good and favorable in green synthesis techniques. This degree of polydispersivity indicates that the nanoparticles are relatively uniform whilst preserving a natural biological variability, which in general can be beneficial to biomedical applications. With a such a distribution, reproducibility and consistency in nanoparticle behavior to various experimental conditions is assured.The DLS data also show a bimodal distribution pattern having the larger fraction of the population and the smaller secondary population of the smaller nanoparticles. Such a dual population is also very advantageous, since the bigger particles help to maintain structural stability, whereas the smaller particles help to enhance surface area and reactiveness significantly. Such an integration enhances the overall functional performance, especially those that are biological, where stability as well as reactivity is a necessity.The fact that the primary peak is always concentrated within a controlled size range further confirms that most nanoparticles are always clustered in a controlled size range. This is the result of effective synthesis and stabilization processes guided by the synergistic effect of the bioactive compounds that are contained in the seed extracts. The nanoparticles boast of strong dispersion properties which reduce the chances of sedimentation thus providing extended stability in the suspension.On the whole, the DLS findings have a high support profile of the successful synthesis of stable, well-dispersed, and biologically compromising zinc nanoparticles. The size distribution is sufficiently small so as to ensure, through sufficient uniformity, that they are sufficiently colloidally stable to be used in more advanced applications in biomedical research. Their properties are especially valuable in the context of azoospermia, since they provide an opportunity to enhance the process of cellular interaction, antioxidant activity, and even support the process of reproductive tissue regeneration.

The dynamic light scattering (DLS) examination of zinc nanoparticles synthesized using fenugreek seed extract show a highly positive and well-defined profile of particle sizes, proving that the synthesis of zinc nanoparticles using fenugreek seed extract was successful. The curve of intensive distribution suggests a predominant and sharp initial peak, which represents the main population of nanuparticles. This high-value peak implies that a large degree of uniformity was achieved in the formation of particles, indicating that the process of biosynthesis effectively controlled the mechanism of nucleation and growth of particles. The measured hydrodynamic diameter indicates an equilibrium state of the nanoparticle system in the presence of water, whereby surrounding phytochemicals cover the particles as a protective layer. This natural capping also contributes to the stability of dispersion, in addition to preventing the uncontrolled aggregation of the nanoparticles, rendering the nanoparticles to be well-suspended in the medium. This stabilization is essential in the regulation of constant physicochemical characteristics and enhancement of the ability to interact with biological systems. The polydispersity index is within a relatively small and healthy range, meaning that the size distribution is relatively small. This implies that the nanoparticles are quite homogeneous with a small variation in size which is a powerful measure of reproducibility and efficiency of synthesis. Such a controlled size distribution can be beneficial in predictable behavior of biologic environments and increases the reliability of the nanoparticles to be used further. According to the DLS data, the bimodal distribution would have a major and a minor peak, which applied to primary population of nanoparticles and a smaller secondary population, respectively. This distribution is beneficial, in that the larger particles help to give overall structural stability, whereas the smaller particles help to increase the surface area and reactivity. Such a balance plays a major role in boosting the functionality of the nanoparticles.The high intensity of the main peak proves that most of the particles are always generated within a controlled size range. This demonstrates the successful use of biomolecules of fenugreek in directing the synthesis and stabilization of nanoparticles. The nanoparticles have a fine dispersion behavior with lower probability of sedimentation, which suggests that the colloidal system is stable.The results of DLS largely confirm the efficient green synthesis of zinc nanoparticles with a high level of stability and uniformity, along with dispersibility features. Numerous positive characteristics present herein underscore their high potential in the biomedical applications namely in regards to azoospermia where better bioavailability, antioxidative activity, and enhanced cellular interaction are critical toward therapeutic efficacy.

CONCLUSION

The present study successfully demonstrates the green synthesis of zinc nanoparticles (ZnNPs) using seed extracts of fenugreek (Trigonella foenum-graecum) and pumpkin (Cucurbita pepo). The use of plant-based extracts provided a simple, cost-effective, and environmentally friendly approach for nanoparticle synthesis, eliminating the need for hazardous chemicals. The bioactive compounds in the seed extracts effectively acted as both reducing and stabilizing agents, leading to the formation of stable nanoparticles. Characterization results confirmed the successful synthesis of ZnNPs with desirable size and morphology. Additionally, the synthesized nanoparticles exhibited notable biological activities, including antioxidant and antimicrobial properties, highlighting their potential applications in medicine, agriculture, and environmental fields. Overall, this study emphasizes the viability of using natural seed extracts for sustainable nanoparticle production and encourages further research to explore their large-scale synthesis and diverse applications.

STATEMENTS AND DECLARATIONS

Funding: Authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests: Authors have no relevant financial or non-financial interests to disclose.

Ethical Responsibilities of Authors: All authors declare that this research article is original and has not been published elsewhere in any form or language (partially or in full). This study has not included any study related to humans and/or animals.

Data transparency: All authors agree that all data, materials as well as software application has supported their published claims and complied with field standards.

Credit authorship contribution statement: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ms. Alshifa Memon (Research scholar) . Dr. Priyanka Singh (as supervisor) and Dr. Yogesh Singh (Co- supervisor) have guided her for designing of experiment, compilation of data and drafting of manuscripts. All authors have read and approved the final manuscript.

Acknowledgements: We are thankful to Nims University Rajasthan to provide lab facility and research support to carry out this research work.

REFERENCES

1. Agarwal, A., Baskaran, S., Parekh, N., Cho, C. L., Henkel, R., Vij, S., & Arafa, M. (2021). Male infertility. The Lancet, 397(10271), 319–333.
2. Aitken, R. J., & Baker, M. A. (2020). Oxidative stress and male reproductive biology. Reproductive BioMedicine Online, 41(3), 617–631.
3. Al-Okbi, S. Y., Mohamed, D. A., Hamed, T. E., & Esmail, R. S. (2019). Pumpkin seed oil and its role in reproductive health. Journal of Food Biochemistry, 43(8), e12919.
4. Bhattacharjee, S. (2016). DLS and zeta potential – What they are and what they are not? Journal of Controlled Release, 235, 337–351.
5. Birkhead, T. R., Hosken, D. J., & Pitnick, S. (2009). Sperm biology: An evolutionary perspective. Academic Press.
6. Brinster, R. L. (2007). Male germline stem cells: From mice to men. Science, 316(5823), 404–405.
7. Bukhari, S. B., Bhanger, M. I., & Memon, S. (2008). Antioxidative activity of extracts from fenugreek seeds. Pakistan Journal of Analytical & Environmental Chemistry, 9(2), 78–83.
8. Durairajanayagam, D. (2018). Lifestyle causes of male infertility. Arab Journal of Urology, 16(1), 10–20.
9. Egerton, R. F. (2016). Physical principles of electron microscopy. Springer.
10. El-Sayed, M. I., & Aly, A. A. (2015). Antioxidant role of fenugreek in reproductive tissues. International Journal of Pharmacy and Pharmaceutical Sciences, 7(4), 13–17.
11. Esteves, S. C., Miyaoka, R., & Agarwal, A. (2011). An update on the clinical assessment of azoospermia. Clinics, 66(4), 691–700.
12. Goldstein, J., Newbury, D. E., Joy, D. C., Lyman, C. E., Echlin, P., Lifshin, E., Sawyer, L., & Michael, J. R. (2018). Scanning electron microscopy and X-ray microanalysis (4th ed.). Springer.
13. Griffiths, P. R., & de Haseth, J. A. (2007). Fourier transform infrared spectrometry (2nd ed.). Wiley.
14. Hafez, E. S. E., & Hafez, B. (2000). Reproduction in farm animals (7th ed.). Lippincott Williams & Wilkins.
15. Honary, S., & Zahir, F. (2013). Effect of zeta potential on the properties of nano-drug delivery systems. Tropical Journal of Pharmaceutical Research, 12(2), 255–264.
16. Howell, S. J., & Shalet, S. M. (2005). Spermatogenesis after cancer treatment. Human Reproduction Update, 11(4), 347–356.

17. Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638–2650.
18. Jarow, J. P., Espeland, M. A., & Lipshultz, L. I. (1989). Evaluation of the azoospermic patient. The Journal of Urology, 142(1), 62–65.
19. Johnston, S. D., Root Kustritz, M. V., & Olson, P. N. S. (2001). Canine and feline theriogenology. Saunders.
20. Kalpana, V. N., & Rajeswari, V. D. (2018). A review on green synthesis, biomedical applications, and toxicity of ZnO nanoparticles. Bioinorganic Chemistry and Applications, 2018, 1–12.
21. King, M. D., & Parry, M. J. (2001). Dynamic light scattering analysis of nanoparticles. Colloids and Surfaces A, 195(1–3), 117–125.
22. Krausz, C., & Casamonti, E. (2017). Spermatogenic failure and genetic defects. Human Genetics, 136(5), 569–582.
23. Kumar, N., & Singh, A. K. (2015). Trends of male infertility. Indian Journal of Medical Research, 142(Suppl 1), S1–S7.
24. Lincoln, G. A., Short, R. V., & Setchell, B. P. (1981). Seasonal breeding and reproductive cycles. Journal of Reproduction and Fertility, 61(1), 1–13.
25. McLachlan, R. I., O'Donnell, L., Meachem, S. J., Stanton, P. G., & de Kretser, D. M. (2002). Hormonal regulation of spermatogenesis. Endocrine Reviews, 23(4), 449–475.
26. Murphy, D. B. (2008). Fundamentals of light microscopy and electronic imaging. Wiley.
27. Nakamoto, K. (2009). Infrared and Raman spectra of inorganic and coordination compounds. Wiley.
28. Nieschlag, E., & Behre, H. M. (2010). Andrology: Male reproductive health and dysfunction (3rd ed.). Springer.
29. O'Donnell, L., Stanton, P., & de Kretser, D. M. (2001). Endocrinology of male reproduction. Endocrine Reviews, 22(3), 289–318.
30. Palmer, N. O., Bakos, H. W., Fullston, T., & Lane, M. (2012). Impact of obesity on male fertility. Human Reproduction Update, 18(5), 553–568.
31. Saeed, F., Arshad, M. U., Pasha, I., Naz, R., Batool, R., Khan, A. A., & Nasir, M. A. (2014). Nutritional and medicinal importance of pumpkin seeds. Journal of Pharmacognosy and Phytochemistry, 3(4), 147–151.
32. Schlegel, P. N. (2004). Causes of azoospermia and their management. The New England Journal of Medicine, 351(24), 2513–2521.
33. Sharpe, R. M., & Irvine, D. S. (2004). Environmental factors and male infertility. BMJ, 328(7437), 447–450.
34. Shokri, S., Aitken, R. J., & Abdolvahhabi, M. (2020). Role of oxidative stress in male infertility. Reproductive Biology and Endocrinology, 18(1), 1–13.
35. Smith, B. (2011). Infrared spectral interpretation: A systematic approach. CRC Press.
36. Stuart, B. (2004). Infrared spectroscopy: Fundamentals and applications. Wiley.
37. Swain, S. S., et al. (2025). Green synthesis approaches in nanotechnology. Journal of Nanobiotechnology, 23(1), 45–60.
38. Wankhede, S., Mohan, V., Thakurdesai, P., & Joshi, A. (2016). Beneficial effects of fenugreek on testosterone and metabolism. Phytotherapy Research, 30(4), 565–571.
39. Wildt, D. E. (2005). Reproductive science in wildlife conservation. Theriogenology, 63(1), 22–35.
40. Yadav, R., et al. (2017). Green synthesis of ZnO nanoparticles using plant extracts. Materials Science in Semiconductor Processing, 66, 1–7.
41. Yousef, M. I., et al. (2019). Role of zinc in male reproductive health. Biological Trace Element Research, 188(1), 1–10.