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Physiological Effects of Green Synthesised Iron Oxide Nanoparticles on the Male Rat Reproductive System

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Abstract | Iron oxide nanoparticles (IONPs) have been increasingly used in a variety of fields, such as biomedicine, cosmetics and medical research. However, information about their possible impacts on human health, particularly reproductive health, is lacking. Our goal is to shed light on the detrimental effects of IONPs on the quality of sperm cells and the reproductive organs of mice, namely, testicular tissues. For 21 days, adult male rats received intraperitoneal injections of green synthesised IONPs made from *Ficus carica* leaf extract. Results showed an increase in testosterone levels, ranging between 5.235 and 8.167 pg/mL. FSH levels were 3.341 – 6.758 IU/L, and LH levels were 5.235–8.346 IU/L, depending on the IONP dose. A decrease in sperm motility (80.5%) and an increase in the percentage of dead sperm (31.4%) were observed compared with those of the control (84.8% and 16.1%, respectively). Additionally, an increase in cell abnormality and total sperm was observed. Histopathological analysis revealed an increase in the number of fully mature spermatozoa situated in the centre of the seminiferous tubule lumen, a change in interstitial tissue indicated by its increased density and a change in the testicle indicated by a high rise in the diameters of the seminiferous tubules and the number of primary and secondary spermatocytes.

Keywords | Iron oxide nanoparticles, Fig leaves, Hormones, Reproductive system, Histopathological

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INTRODUCTION

Iron oxide nanoparticles (IONPs) have many biological applications including drug delivery, gene delivery, cell tracking, hyperthermia, and use as a contrast agent in MRI (Albukhaty *et al*., 2024; Saod *et al*., 2024; Khaleel *et al*., 2024). Iron nanoparticles (NPs) are used as food supplements when used in human food, like iron-fortified drinks and cereals (Chavarría-Fernández *et al*., 2024). They are also used for a variety of industrial purposes, including gas sensing, wastewater treatment, semiconductors, sorbents, lubricants, pigments and coatings (Aragaw *et al*., 2021). Regardless of IONP's potential benefits, it is a prerequisite that they are validated to not cause any cellular damage (Siddiqui *et al*., 2023). IONPs orbital impacts on health have been a popular subject for research due to the number of different ways that IONPs can enter the body and as they can be ingested, inhaled and absorbed to the skin (Lewinski *et al*., 2008).

Some NPs can cross biological barriers and harm essential organs, including kidneys, liver and brain. After being intravenously injected, silver NPs initially accumulate in organs including the liver, lungs and spleen. Similarly, a

single intravenous injection of gold NPs can lead to their prolonged accumulation in the liver and spleen. The slow accumulation of NPs in the kidneys, blood and testes and their gradual decline in urine, faeces and lungs suggest their ineffective elimination and potential redistribution within the body (Magdy *et al*., 2022). The kidneys and liver are the major organs where NPs tend to accumulate, regardless of the exposure route, animal model or NP physicochemical properties (Waris *et al*., 2023). NPs can accumulate in reproductive organs and pass through specific biological membranes. Sundarraj *et al*. (2017) indicated that 50 nm of magnetic NPs might cross the mouse blood-testis barrier and land in the reproductive organs.

The interaction of biological processes of these NPs can influence environmental exposure and the potential for cellular uptake raises alarms about concerns related to reproductive toxicity (Samrot *et al*., 2023). Reproductive toxicity has become recognized as an important facet of general toxicology requiring additional research (Miller *et al*., 2024). Fertility and the possibility and success of reproduction is essential to survival for a species, therefore educating the public regarding NP-associated reproductive toxicity is warranted because the possibility of custom NP development is always increasing, which heightens the odds and potential impact that NPs could disrupt the reproductive system (Hong *et al*., 2023). The male reproductive system is susceptible to oxidative stress and inflammation both of which can be indicators of exposure to NPs (Walke *et al*., 2023) and either one may be factors in NP induced reproductive toxicity (Samrot and Prakash, 2023). Oxidative stress is a prominent factor in NP induced reproductive toxicity; it has been observed and noted that reactive oxygen species (ROS) contribute to 30%–80% of male infertility related issues, ROS is responsible by excessive induction of cell death and impaired spermatogenesis (Iftikhar *et al*., 2021).

The present study aimed to investigate the effects of IONPs on adult Wistar rats sperm quality, fertility and reproductive hormones.

MATERIALS AND METHODS

Preparation of the extract

After being cleansed twice with tap water, freshly harvested *Ficus carica* leaves were rinsed with deionised water and dried in an oven set at 70 °C for 2 days. The dried leaves were ground into small pieces using a laboratory grinder. Volume 200 mL of deionised water was used for dissolving 10 g of the finely chopped leaf particles in a 500 mL flask with a flat bottom. For an hour, the mixture was heated and swirled in a water bath set at 80 °C. The aqueous leaf extract was filtered using filter paper, placed in an amber bottle and kept in the refrigerator until use (Sandhy and Kalaiselvam, 2020).

Green synthesis of IONPs

Briefly, 0.01 M (2.70 g) of FeCl3.6H2O was dissolved in 100 mL of deionised distilled water to create IONPs. The mixture was placed in a conical flask and heated to 70 °C under a mechanical stirrer. Then, 40 mL of *F. carica* extract was gradually dropped into the previous mixture. The mixture was added with NaOH (0.1 M) to increase the pH to 11 and then placed on the stirrer for 1 h. After centrifugation, the resultant mixture produced a precipitate that was repeatedly rinsed with H2O to eliminate metabolites and unreacted salt. The precipitate was after that dried in an oven set at 80 °C for one day (López-Alarcón *et al*., 2011; Kamath *et al*., 2020).

Characterisation of NPs

The following instruments were used to characterise the resultant IONPs: A Shimadzu IR-Affinity spectrophotometer for FTIR spectroscopy, a Shimadzu UV-1800 for UV–vis spectroscopy; a Rigaku RINT-2000 for measuring XRD patterns; and SEM (Supra 50 VP) and dynamic light scattering (DLS) for analysing particle morphology and size.

Experimental animals

Twenty-one adult male rats were used in the experiment and divided into three experimental groups of seven rats each. A low dose of IONPs (50 mg/kg) was given to Group 2, a high dose of IONPs (150 mg/kg) was given to Group 3. Group 1 was designated as a control group that received distilled water. Following 21 days of injection with the aforementioned medications, the animals were not given any more doses for 2 days. Chloroform was used to put the animals to sleep on the 3rd day. The animals were stabbed in the heart (cardiac punctured) to obtain their blood. The blood samples were centrifuged for 10 min at 3500 rpm to rapidly obtain blood serum. Prior to biochemical testing, the serum was kept at –20 °C. For the preparation of histological sections, the animals were dissected and the testes were taken out and kept in a 10% formalin solution as a fixative.

Serum hormone analysis

With the use of the kits provided by SunLong Biotech Co., Ltd., the sandwich ELISA technique was used to measure the levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the sera taken from each group.

Sperm motility

For the estimation of sperm motility rate, the tail of the epididymis was placed in a glass dish on a hot plate (37 °C)

containing a physiological saline solution and then cut to release the sperm. A drop of the solution was then taken and placed on a slide with a cover slide. Sperm movement was observed under a light microscope at 40× magnification (Dehghan *et al*., 2005).

Sperm abnormality

Live and dead sperm counts were used to calculate the percentage of deformed sperm. Head, neck and tail deformities were observed under a light microscope at 40× magnification. The percentage of deformed sperm was calculated using the following formula:

Percentage of deformed sperm= (Number of deformed sperm/ Number of total sperm) x 100

Percentage of dead sperm

A drop of the mixture containing the sperm was placed on a slide fixed on a hot plate at 37 °C, followed by another drop containing the necrocin-eosin mixture. The two drops were mixed with a clean glass slide, and the mixture was drawn along the length of the slide and left to dry at 40 °C.

Percentage of dead sperm= (Number of dead sperm/Number of total sperm) x 100

Sperm count

The decapsulated left testis was used to determine the epididymal sperm count. The head and body plus tail were the two sections of the left epididymis. For 20 min, the two components were mixed in 50 mL of a solution containing 0.05% Triton X-100, 0.01% merthiolate and 0.9 NaCl. The sperms were counted using a haemocytometer (Kingsley *et al*., 2015).

Histological evaluation of testes

The testicles were histologically assessed using the technique of Abdollahi *et al*. (2023).

RESULTS AND DISCUSSION

Characterisation of IONPs

The characteristics of IONPs can be determined by examining their spectral profile and the results of other characterisation tests. UV–vis spectroscopy is a reliable and constructive method to characterise generated NPs and monitor their stability and synthesis (Abdussalam-Mohamed *et al*., 2022). Iron NPs display an absorption peak at 300–500 nm; this peak shift supports the nanoscale nature of IONPs with a core-shell form. Tauc plots are frequently used to measure the optical bandgap of materials, such Fe2O3 (hematite) NPs. The y-axis indicates (αhv)2, and the x-axis depicts photon energy (hv) in electron volts (eV). An increase in optical band gap was observed relative to that of bulk structures. This phenomenon can be attributed to the shift of the absorption edge to lower wavelengths due to the decrease in particle size related to the NPs' structural characteristics (Figure 1).

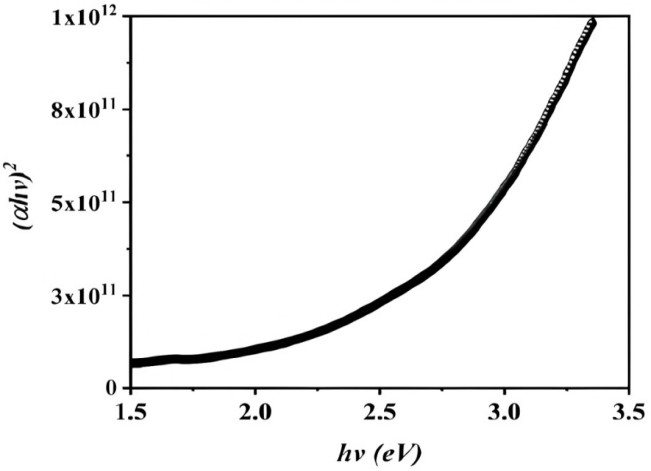

Figure 1: Absorbance of IONPs.

Figure 2: Size of IONPs determined using SEM.

Figure 2 shows the morphology of the NPs, which appear roughly spherical and densely packed across the whole surface with a size of approximately 20–30 nm. A rod-like structure can be found in the lower right. SEM results show spherical or slightly irregular shapes with a uniform particle distribution, which is crucial for consistent interaction with biological tissues. Figure 3 is the AFM image showing the 3D surface morphology of Fe2O3 nanostructures. The height scale (colour bar on the right) extends from 0 to ~38 nm, indicating surface roughness and NP distribution which govern the biological system's material interactions, wettability and catalytic activity.

The FTIR spectroscopy in Figure 4 reveals the functional groups involved in the synthesis of IONPs from fig leaf extract. The FTIR spectra exhibit peaks related to polyphenols, which act as reducing and stabilising agents. These peaks typically include the characteristic peaks for

hydroxyl (-OH) groups, carbonyl (C=O) stretches and possibly C-H or C-O bonds. These signals confirm the successful capping of iron NPs by organic compounds from fig leaves, which enhance biocompatibility.

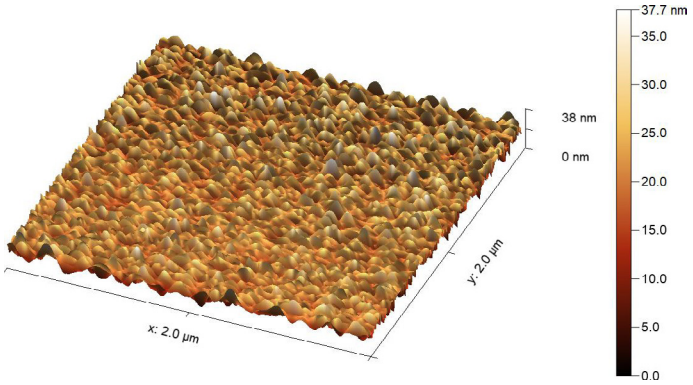

Figure 3: AFM distribution and size of IONPs

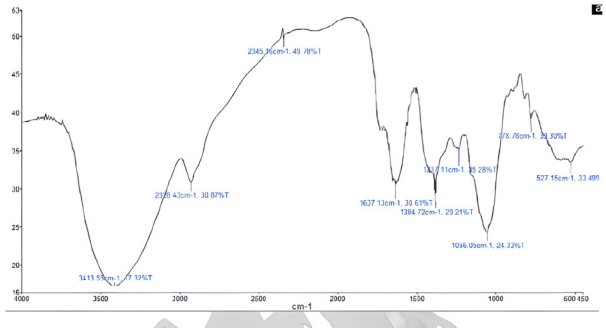

Figure 4: FTIR of IONPs

Figure 5 displays the X-ray diffraction spectrum of Fe_2O_3NPs. The peaks at (220), (311), (400) and (422) correspond to the diffraction planes of α-Fe2O3 (hematite). The prominent peak at (311) 30° indicates a well-crystallised structure. Multiple peaks confirm the purity and crystallinity of Fe2O3 NPs.

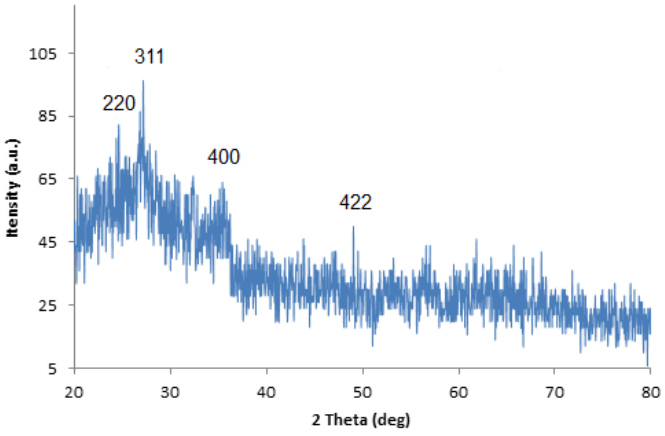

Figure 5: XRD pattern of prepared IONPs

Serum hormone results

IONPs have become a major global issue because of their superparamagnetic physiochemical characteristics and possible biomedical uses. The function of IONPs in maintaining hormonal balance in male rats was investigated in this work. Table 1 shows that testosterone, FSH and LH levels were higher in the IONP-treated groups (50 and 150 mg/kg) than in the control group. This finding is consistent with the research of Kamel and Al-Taee (2020), who found that treating male and female rats with IONPs increased the levels of reproductive hormones such as LH, FSH and testosterone. Following this rat exposure to MoO3 NPs, another investigation by Magdy *et al.* (2022) showed a substantial increase in LH and FSH levels. The possible impacts of NPs on functional cells could result in a defect in their secretory activity and an imbalance in normal sex hormone levels. Furthermore, NPs increase and release ROS, which could promote the oxidation of cellular macromolecules such as proteins (Wang *et al.*, 2024).

Table 1: Effect of IONPs on male reproductive hormones.

LH (IU/L)	FSH (IU/L)	Testosterone (pg/ml)	Treatment
3.620	3.341	4.23	Control
5.235	4.230	5.235	Fe Nps 50 mg/kg
8.346	6.758	8.167	Fe Nps 150 mg/kg
1.54	2.430	1.345	LSD 1%

Effect on sperm vitality

Table 2 shows a decline in sperm motility with the increase in abnormal sperm count and number of dead sperm upon exposure to IONP doses of 50 and 150 um/kg compared with that of the control group. This result is consistent with Younus *et al.* (2020), who examined sperm count and sperm motility in mice given doses of iron oxide nanoparticles (IONPs) for 5, 10, 20, and 40 mg/kg. There was a decrease in sperm motility in the epididymis between the experimental and control groups. Iron nanoparticles could cross sperm membranes and target the mitochondria and acrosome (Nasri *et al.*, 2015). It is also noted that sperm motility was observed in the epididymis. Iron NPs may affect the epididymis and cause inflammation which would reduce motility of sperm. Additionally, iron NPs may increase the level of reactive oxygen species (ROS) that affect the flagella structure and motility of sperm (Vassal *et al.*, 2021). An increase in ROS concentration could be caused by various metal NPs including superoxide which could inhibit RNA polymerase (Javaid *et al.*, 2025).

Table 2: Effect of IONPs on sperm counts.

Count %	Abnormal %	Dead %	Motility %	Treatments
21.3	15.3	16.1	84.8	Control
23.5	18.6	22.6	75.6	Fe Nps 50 mg/kg
26.3	22.2	31.4	80.5	Fe Nps 150 mg/kg
2.47	4.236	9.43	12.30	LSD 1%

Table 3: Effect of IONPs on testicular cell counts and seminiferous tubule measurements.

Parameters/ Treatments	Seminiferous tubules in microscopic field (µm)	Seminiferous tubules in microscopic field (10×)	Leydig cells (Number)	Sertoli cells (Number)
Control	300±2.47	22.45±2.97	39.25±2.42	26.95±1.59
Fe Nps 50 mg/kg	205.6±10.08	11.25±1.924	22.65±5.39	17.25±1.924
Fe Nps 150 mg/kg	399±8.34	20 ±2.24	36.25±1.7	25.65±2.39
LSD 1%	21.43	7.55	7.65	8.22

Histopathological studies

Upon the administration of IONPs at a dosage of 150 um/kg, histopathological study (Table 3 and Figure 6) reveals a reduction in testicular cell counts and seminiferous tubule measures against the control. The diameter of seminiferous tubules, interstitial tissue thickness, interstitial cell count, and distance between seminiferous tubules were impacted as well. The increase in NPs found in testicular tissues, including Sertoli cells, Leydig cells, and spermatids, may be behind these effects. Different molecules, including DNA and proteins, could oxidise when exposed to this concentration. This oxidative stress might reduce the number of Leydig and Sertoli cells (Mirzaei Varzeghani *et al*., 2018). Leading cells' DNA could be damaged by IONPs, causing their immediate or eventual death (Dantas *et al*., 2024). Reacting iron NPs with DNA causes oxidative stress, inflammation and cell function loss (Cameron *et al*., 2022). By breaching the blood barrier in testicular tissues, damaging the sperm cells and influencing their stem cells, IONPs exert a harmful effect on the male reproduction system (Habas *et al*., 2021; Rossner *et al*., 2023). Our results underline how IONPs negatively affect sperm. We propose that the molecular mechanism occurs in Leydig cells.

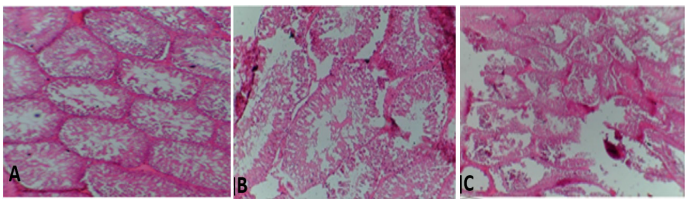

Figure 6: Effects of iron nanoparticles on the testis as observed under a light microscope at 40× magnification. (A) control, (B) dose 50 mg/kg and (C) dose 150 mg/kg.

CONCLUSION

IONPs at were produced by the biological materials found from the *Ficus carica*. The height scale AFM (colour bar on the right) extends from 0 to ~38 nm, indicating surface roughness and NP distribution which govern the biological system's material interactions, wettability and catalytic activity. NPs increase and release ROS, which could promote the oxidation of cellular macromolecules such as proteins Iron oxide nanoparticles could increase sex hormone levels and sperm production in rats. They could also harm testicular cytology and damage to the tissue under study this may be led to reproductive toxicity. Evaluating the long-term consequences and underlying causes of these developments is necessary. Our results underline how IONPs negatively affect sperm. The molecular mechanism occurs in Leydig cells were propose.

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NOVELTY STATEMENT

This study presents a new approach using iron oxide nanoparticles (IONPs) extracted from fig leaves to investigate their effects on the reproductive system in rats. The results revealed harmful effects of iron oxide nanoparticles on sperm quality and reproductive organs in rats, specifically testicular tissue.

AUTHOR'S CONTRIBUTION

FJA-O, ERAG and NIL: Significant contributions to this work.
FJA-O: Responsible for the study design.
NIL: Performed the experiments and collected the data.
ERAG: Contributed to the data analysis and interpretation of the results.
FJA-O and ERAG: Drafted the manuscript.
All authors read and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no known personal, financial, or other conflict of interest that might influence the work reported in this manuscript.

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