



Spermicidal Activity of Gold Nanoparticles on Male Mice *in Vitro*

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Abstract | The effect of Gold Nanoparticles (GNPs) on sperm concentration and motility was studied in the acute toxicity of Gold nanoparticles on semen suspensions using MTT, revealing an increase in sperm death markers with rising Gold nanoparticles concentrations. The lethal concentrations of Gold nanoparticles were determined, with LC_{50} and LC_{95} found to be 176.48 and 349.63 respectively. The study examined sperm motility, showing a significant variation at the onset of recording motile sperm, suggesting that sperm concentration was dependent on turbidity. There was a positive correlation between the delay at the start of absorbance recording and Gold nanoparticle exposure concentrations. However, the overall population of motile sperm showed a negative correlation with increased Gold nanoparticle concentration. The Gold nanoparticle concentrations effectively hindered the progressive motility of sperm. Lag time results indicated decreased time values with increased Gold nanoparticle concentrations, suggesting a negative prognosis. The velocity of sperm within a semen suspension exhibited a marked decrease as the concentration of gold nanoparticles increased. Concurrently, The Fraction of Rapidly Moving Sperm was reduced with increasing Gold nanoparticle concentrations, indicating a negative correlation. The motility index of sperm was notably influenced by the presence of gold nanoparticles, exhibiting a pronounced decline as the concentration of these nanoparticles escalated. The study investigated the impact of Gold nanoparticles on DNA abnormalities. The concentration of Gold nanoparticles increased; there was an increase in histone residues and abnormal DNA chromatin, indicating a concentration-dependent relationship. These findings highlight the potential deleterious effects of Gold Nanoparticles on sperm motility and DNA integrity. The study concluded that Gold nanoparticles affect sperm concentration and motility, with increased Gold nanoparticle concentrations leading to higher sperm death markers and reduced motility. An observed correlation exists between the concentrations of gold nanoparticles and the incidence of DNA abnormalities; elevated levels of gold nanoparticles are associated with an increase in histone residues and anomalies in DNA chromatin structure.

Keywords | Gold nanoparticles, Sperm, MTT, LD_{50} , Acute toxicity, Lag time, Motility index, DNA, Acridine, X-ray diffraction, Alanine blue

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INTRODUCTION

A spermicide is a chemical substance revealed to destroy or immobilize sperm in the vagina and act as a con-

traceptive effect on the female genital tract. Thus, the spermicide mode of action ranged between disruption and distorting sperm physiology activity in topical application, as well as causing sperm mortality in the vaginal route, which

The problematic issue with conventional contraceptive methods lies in their reliance on hormonal manipulation, which disrupts physiological hormonal equilibrium and may signal various systemic conditions. These methods can interfere with normal ovum production and induce substantial alterations in the endometrial lining. All the pharmaceuticals surveyed to be used as plane infertility inducers in male gametes or as spermicides into the vagina-cervical canal were not sufficiently efficient, in addition to not having complete safety due to low selective toxicity or low efficiency as a barrier with their adverse effect outcomes in female (Hifnawy *et al.*, 2021). Moreover, a reduction in testosterone production (Liu *et al.*, 2020) was observed. Regrettably, the reversibility of GNP-induced reproductive toxicity was not explored in those particular studies. For this reason, Gold nanoparticles were chosen and challenged due to their capability of penetrating inside the cells and combining with DNA molecules due to their extremely small sizes and unique physical and chemical properties sperm quality and quantity may interfere. Several Notable Research on the ability of GNPs with different sizes in penetration into the DNA molecule and positioning in DNA large grooves has been shown (Appasamy *et al.*, 2007). This evidence that certain nanoparticles exhibit spermiotoxic properties, potentially inhibiting sperm motility and hindering sperm chromatin decondensation (Wiwanitkit *et al.*, 2009). This research paves the way for the development of effective spermicides in animals and the exploration of targeted actions as concentrations change. The study aimed to modify GNP activity on semen suspension and reset the efficiency concentrations of prepared heparinized Gold nanoparticles as a spermicidal agent.

MATERIALS AND METHODS

The design, technique, and protocols were based on the usage and care of laboratory animals in the teaching guide and research (AUCAC) (Al-Bayati and Khamas., 2015) and were documented by the Graduated approval system of Iraq's College of Veterinary Medicine and approved by document report No. 2336 and dated 14/12/2023: University of Baghdad - College of Veterinary Medicine.

PREPARATION AND STANDARDIZATION OF HEPARINIZED GOLD NANOPARTICLES

Several steps were provided as a sequence step for the achievement capping initiator for synthesis GNPs and stabilizing the preparative methodology was run as flow steps (Vidya and Saji, 2018). The stock solution of AuCl₄ was processed by dissolving 0.67g of Auric chloride in 100 ml deionized water to achieve 0.02M expressed as 0.67%. Auric chloride 1ml was transferred to a conical flask (50ml) and mixed with 4.5ml of deionized water 0.148 %. The

working diluted AuCl₄ 5.5 ml was boiled at 95°C for 45 minutes with stirring at 500 rpm/ min on the magnetic stirred-hot plate. Heparin 10ml (5000 IU) was dropped on boiled Auric chloride gradually; to prevent clumping, with stirrings until the yellow solution became a red-brown Nanoparticles solution (Vidya and Saji, 2018). Forming Gold nanoparticles were stabilized by adding Hydrochloride acid 1ml, 1n for stabilizing and increasing the reducing rate (King *et al.*, 2015).

STANDARDIZATION OF GNP

The physicochemical properties of the Nano-gold particles were characterized using various techniques. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) were employed at the Alkhora company's Nano research lab. Dynamic light scattering (DLS) and zeta potential measurements were performed at the Ministry of Science and Technology laboratory, while atomic force microscopy (AFM) analysis was conducted at the Chemistry Analysis Centre.

EXPERIMENTAL DESIGN AND PROTOCOL

The thirty mice semen suspension was grouped first as the control group, and second Gold Nanoparticles (GNP) group: The treated GNP groups were divided into five sub-groups according to exposure concentrations of Heparinized-Gold Nanoparticles in semen suspension and set as follows: 100, 150, 200, 250, and 300 µg/ml modified from (Batoool *et al.*, 2022), for achieved Log concentrations Response curve.

ANIMAL

The mice were obtained from the Industrial Development Al-Razi Center for Research and Medical Diagnostic Kits productions animal house: Corporation for Research and Industry Development, Ministry of Industry and Minerals, the mice were left for one week for adaptation, and supplied free tap water and *ad libidum* concentrated meal, the room was controlled temperature (25±2 C°), humidity and ventilation.

SPERM SUSPENSION PREPARATION

The male mouse was euthanized via inhalation of diethyl ether. It was necropsied for the excision of the epididymis (Martinez, 2022). The cauda of the epididymis was separated and The caudal of the epididymis was weighed. each cauda epididymis was placed in 0.5 ml of Dulbecco's Modified Eagle Medium (DMEM) at 37 C° in a pre-warmed glass watch. and then minced 200 times with microsurgical scissors (Abd-alhussein and Al-Bayati, 2021). Before proceeding with the experimental protocols, the individual motility of the stock semen suspension for each mouse was checked to confirm the healthy functioning of the sperm (Hadi, 2013a). Sperm concentration and viability were assessed using a standard curve generated by serial dilutions

of sperm suspensions in Dulbecco's Modified Eagle Medium (DMEM). Aliquots containing 20, 30, 40, 50, and 60% of the calculated sperm count (determined using a hemocytometer) were prepared. Absorbance was then measured at a wavelength of 450 nm using a spectrophotometer, then blotted best-fitted line versus concentrations (Brito *et al.*, 2016). As well as The determination of viable sperm percentages at dilutions of semen suspension 2×10^2 , 2×10^3 , 2×10^4 , 2×10^5 and 2×10^6 per ml. A 50 trypan blue was added to 0.1 ml of each concentration of semen and examined the slide under a light microscope at 100 X magnification. Then semen suspension was checked MTT, the MTT method was placing 0.1 ml of semen of each concentration on the microliter plate well, and then 0.1 of MTT was left mixture for 15 minutes at room temperature and the color-forming was estimated at wavelength 450 nm in spectrophotometers. The viable sperm was plotted with absorbance values for each concentration of semen suspension as a calibrated curve of viability (Strober, 2015).

ACUTE TOXICITY (LCS) ON MICE SEMEN SUSPENSION

According to (Finney, 1971) the acute toxicity of Gold Nanoparticles in semen suspension 2×10^6 sperm/ml was determined by the calculation of LCs (LC_{50} and LC_{95}) by determining via MTT on 0.1 ml GNPs concentrations 0, 100, 150, 200, 250, and 300 ug/ml. The semen reaction was diluted with 1.7ml normal saline and transferred to a cuvette for determination of the absorbance at wavelength 450 nm.

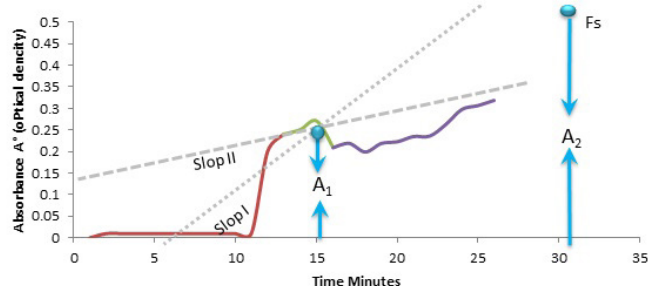


Figure 1: Turbidimetric analysis of sperm motility plotted a graph of sperm kinetic sperm motility modified from (Al-Bayaty, 1999) A_1 : the distance from the x-axis to the point of cross between slop tends of a fraction of rapidly moving sperm and plateau maintenance sperm curve A_2 : the maximum distance between the axis to the point of absorbance of mixed semen suspensions. Slop 1: The slop one Ravidly Moving Sperm. Slop 2: Ordinary sperm moving. F_s : The total sperm count in the whole sample (sperm/ ml).

TURBIDIMETRIC ANALYSIS OF SPERMS MOTILITY

According to (Sakolowski *et al.*, 1977; Al-Bayaty, 1999), the spectroscopic Turbidimetric method of sperm motility analysis was used to describe the motility indices; this approach was assessed as follows: a masked cuvette narrow

window containing 1 ml of normal saline was filled with a 50 μ l of diluted semen suspensions. Sperm motility was assessed using spectrophotometry. The motility of sperm was recorded as sperm kinetic and traced curve absorbance versus time for each GNP-treated semen suspension and control. The kinetic parameters of motility were determined as follows (Figure 1). Log-transformed dose concentrations were plotted against motility indices. Half-maximal effective concentration (EC_{50}) and the maximal effect (E_{max}) were calculated using a fitted curve, following the method described by (Al-Bayaty, 1999). The parameters of sperm motility analysis were:

LAG TIME (SEC.): The lag time refers to the time between injecting diluted semen suspension and the first rise in absorbance, indicating the active injection port. The quickest fraction of sperm to exit the sample is 4 mm in our equipment, with a bottom window of 1 cm (Hadi, 2013b).

FRACTION OF RABIDLY MOVING SPERM (FRMS): The percentage of fast-traveling sperm was discovered by Equation 1.

SPERM MOTILITY INDEX (SMI) %: (Ibrahim and Al-Bayati, 2014) the total motile of all types of wave's motility in semen samples of whole total viable sperm percent Equation 2.

THE VELOCITY OF FRACTION RAPIDLY MOVING SPERM (FRMS): According to (Al-sabaawy *et al.*, 2020) the speed of sperm per unite time of elite rapidly moving sperm was determined via Equation 3.

$$FRMS \text{ (sperm /ml)} = \frac{A_1}{F_s \cdot A_2} \text{Equation 1}$$

$$SMI \% = \frac{\text{slop } A_1}{\text{caudal weight (Total sperm count} \times \text{Viability)}} \times 100 \text{Equation 2}$$

$$VRM \text{ um/sec} = \frac{\text{slop}}{A_1} \text{Equation 3}$$

DNA ABNORMALITY: The DNA abnormality test for assessment of chromatin quality via different vital staining methods according to (Talebi *et al.*, 2007; Talebi *et al.*, 2011) and explored 1. DNA Integrity and Condensation: Toluidine Blue Dye (TB) 2. Abnormal Sperm DNA: Acridine Orange (AO) 3. Histone Residue: Aniline Blue (AB).

STATICAL ANALYSIS

Data were analyzed for mean and standard error. One-way and two-way analysis of variance (ANOVA) with F-test was used to identify statistically significant differences. The least significant difference (LSD) post-hoc test was employed to determine specific group differences. Dose-response curves were generated using Microsoft Excel 2010.

The best-fit line was calculated for linear and quadratic equations. Lethal concentration (LC) values were determined using the Probit method within Microsoft Excel 2010, as described by (Steel and Torrie, 1981).

RESULTS AND DISCUSSIONS

STANDARDIZATIONS OF HEPARINIZED GOLD NANOPARTICLES

Gold nanoparticles synthesized the color change from yellow to red-brown after 45 minutes at boiled degree 95 C° depicted in Figure 2.

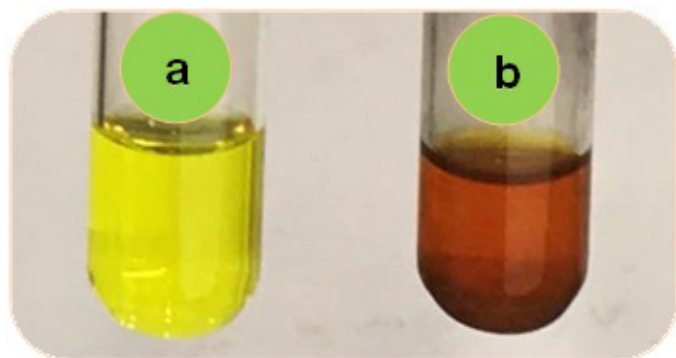


Figure 2: a: auric chloride working solution; b: Heparinized GNP red-brown color.

SCANNING ELECTRON MICROSCOPE (SEM): The morphology and size of Gold nanoparticles in Figure 3, showed the heparinized GNP shape as a cubic cage box.

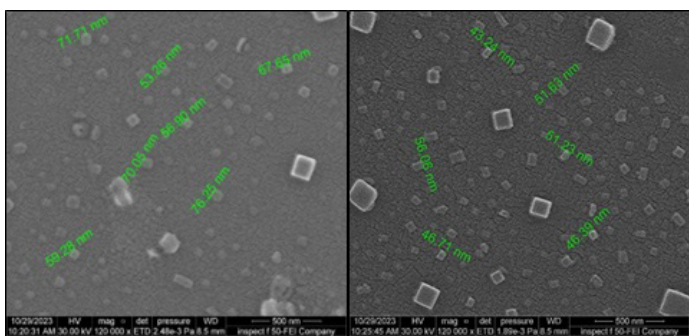


Figure 3: Scanning Electron Microscope (SEM) Micrograph of GNP Sample.

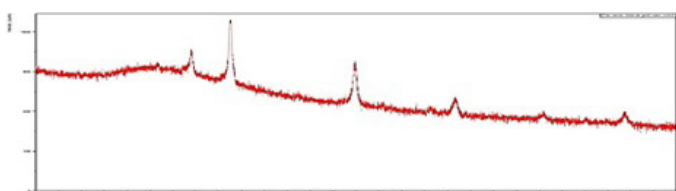


Figure 4: X-ray pattern of Gold Nanoparticles and peak revealed crystalline.

X-RAY DIFFRACTION (XRD): The X-ray diffraction was performed to establish conformation of the GNP composition with the presence of other elements as well

as the crystalline nature of heparin-GNPs the pattern of analyzed GNP showed diffraction peaks were recorded from 15° to 75° at 2 theta angles. the diffraction peaks 23.31°, 27°, 31.30°, 53.12°, 55.89° and 74.30° as a reflection of metallic Gold The diffraction peaks of all peaks corresponded to the (177), (445), (405), (145), (298) and (331) lattice planes in Figure 4.

ZETA POTENTIAL: The colloidal stability of GNPs was assessed using zeta potential to evaluate their surface charge. The analysis revealed a surface charge of -17.09 mV on the GNPs, indicating sufficient electrostatic repulsion for stability. A decrease in zeta potential is observed in Figure 5.

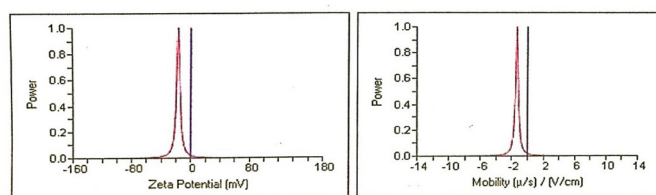


Figure 5: The surface charge and stability of Gold Nanoparticles by zeta potential.

ATOMIC FORCE MICROSCOPY (AFM): Analysis was used to determine the surface morphology of Gold nanoparticles. GNPs showed heterogeneity of shape GNPs with a thickness range from 5.809 nm to 67.62 nm had a surface cubic to triangular Nanoparticles shape had a surface distance of 11.61 nm and vertical distance of 9 nm as well as a fraction number of size (Figure 6).

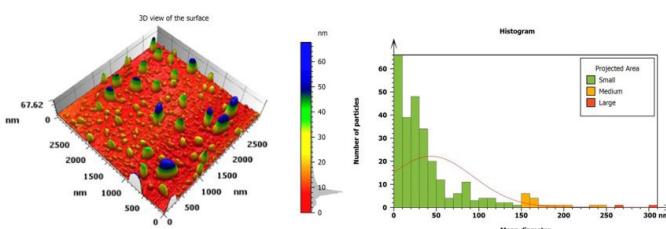
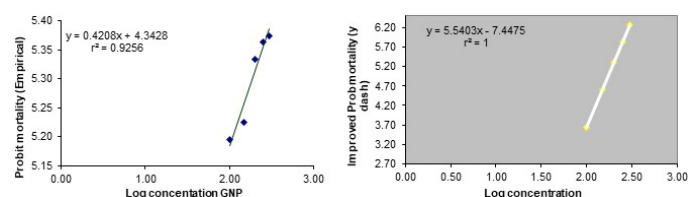


Figure 6: The range thickness, surface distance, and vertical distance of GNPs of (AFM).

GOLD NANOPARTICLES LETHAL CONCENTRATIONS OF SPERM

The acute toxicity of Gold nanoparticle concentrations (GNPs) challenged by serial concentrations on semen suspensions (2×10^6) with serial concentrations of GNPs were tested by MTT, the graduation of GNPs increment concentrations 100, 150, 200, 250, 300 $\mu\text{g/ml}$ exhibited increased the percentage of MTT absorbance value indicative to increase sperms death markers 1.4654, 1.4854, 1.5568, 1.5754 and 1.582 respectively. The dose-response relationship between GNP concentrations and Probit lethal responses exhibited a sigmoidal curve with a lower plateau, characteristic of threshold responses (Figure 7). The

median lethal concentration (LC₅₀) and lethal concentration for 95% mortality (LC₉₅) of GNPs were 176.48 and 349.63, respectively.



Figures 7: acute lethal curve of Gold Nanoparticles: a. Relationships between log concentration of Gold Nanoparticles and Probit mortality. B. Relationships between log concentration of Gold Nanoparticles and Probit mortality.

SPERM MOTILITY ANALYSIS

Figure 8 presents a schematic representation of the Turbidimetric analysis of sperm motility. The experiment involved groups treated with varying concentrations of (GNP) - Control, 100, 150, 200, 250, and 300 µg/ml. Changes in sperm suspension absorbance over time exhibited a positive correlation with motile sperm density, which was recorded over time, showed an upward trend corresponding to the density of motile spermatozoa. The overall sperm curve indicated a significant variation ($p \leq 0.05$) at the onset of recording motile sperm, suggesting that sperm concentration was dependent on turbidity. There was a significant positive correlation ($p \leq 0.05$) between the delay at the start of absorbance recording and GNP exposure concentrations. Moreover, the motility slopes deviated and shifted to the left, depending on the GNP concentrations. This shift was more pronounced in certain treatment groups, showing a negative correlation between the overall population of motile sperm and increased GNP concentration. The final analysis of sperm motility focused on two specific concentrations of treated semen: 250 µg/mL and 300 µg/mL. These concentrations effectively hindered the progressive motility of sperm during the screening process.

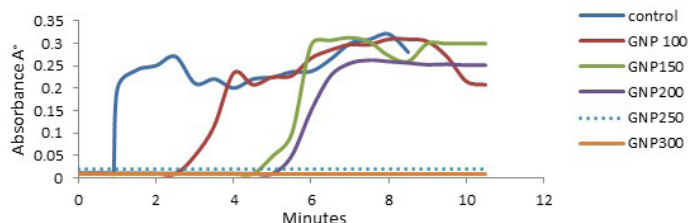


Figure 8: denoted to the timeline of motile sperm kinetic movement for serial exposure to GNP concentration, the non-waves lines in the curve referred to zero value of absorbance of immotile factions of semen.

LAG TIME

The quantitative linear movement of ascending upward of progressive sperm achieved through Lag time results re-

ferred to decreased time values with increased concentration of GNPs as a negative prognosis significant ($p \leq 0.05$) as compared with control and between GNP treated semen suspension groups followed increased to GNP concentrations attributed to dependent on initiation of fraction rapidly moving sperm progressive motility waves in (Figure 9) and displayed Lag time 0.9 ± 0.005 , 2.5 ± 0.03 , 4.5 ± 0.06 , 5.1 ± 0.34 , 0 ± 0 , 0 ± 0 minutes for control and exposure concentration, 100, 150, 200, 250, and 300 µg/ml respectively, which significant ($p \leq 0.05$) increase as increase concentration of GNPs (dose-dependent). The GNP ECs of Lag time EC_{max} = 277.9.

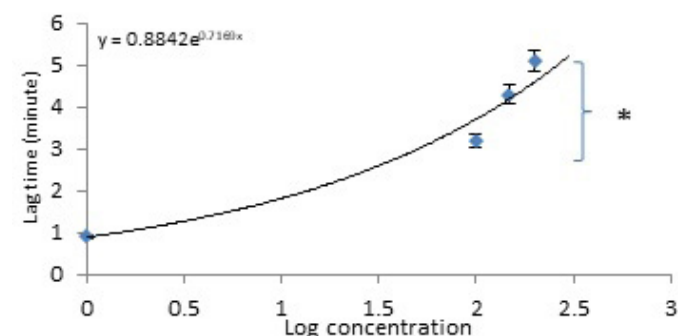


Figure 9: The Log concentrations Lag time (Minutes) relationship curve of mice sperm challenged Heparinized Gold Nano-particles. The data presented as SE $\pm$ Mean, the stars denoted to significant $p \leq 0.05$. n=5 mice semen suspensions.

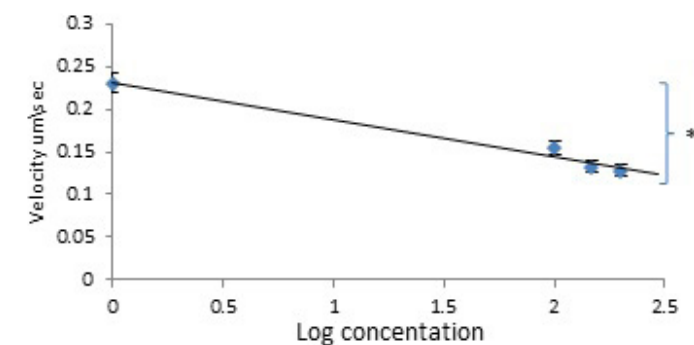


Figure 10: The Log concentrations velocity relationship curve of mice sperm challenged Heparinized Gold Nano-particles. The data presented as SE $\pm$ Mean, the stars denoted to significant $p \leq 0.05$. n=5 mice semen suspensions.

THE VELOCITY OF THE FRACTION OF RAPIDLY MOVING SPERM $\mu\text{M}\text{SEC}$

Sperm velocity in the semen suspension exhibited a typical decline with increasing concentrations of GNPs (Figure 10). This figure illustrates the relationship between sperm velocity of mice semen suspension and exposure to increasing concentrations of GNPs (0, 100, 150, 200, 250, and 300 µg/mL). The percentage of the velocity of the fraction of rapidly moving sperm for each GNP concentration is presented (0.231 ± 0.021 , 0.155 ± 0.006 , 0.133 ± 0.002 ,

0.128±0.008, 0±0 and 0±0 µm/sec) significant ($p \leq 0.05$) reduction as compared with control significant and $EC_{50} = 15.84$ µg/ml $EC_{max} = 245.47$ µg/ml.

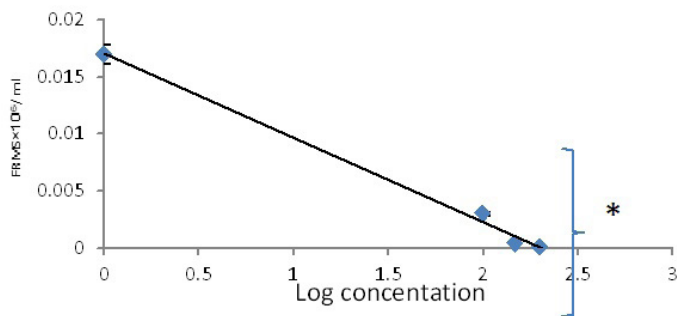


Figure 11: The Log concentrations fraction rapidly moving sperm relationship curve of mice sperm challenged Heparinized Gold Nano-particles (FRMS: Fraction of Rapidly Moving of Sperm) The dated presented as SE±Mean, the stares denoted to significant $p \leq 0.05$. n=5 mice semen suspensions.

FRACTION OF RAPIDLY MOVING SPERM (FRMS)

The FRMS $\times 10^6$ per ml in the figure represented the elite sperm having a high-velocity fraction of sperm. (Figure 11) showed and displayed the negative correlation between FRMS and Gold Nanoparticles significant ($p \leq 0.05$) reduction of FRMS at concentrations 100, 150, 200, 250, and 300 µg/ml as compared with control semen suspension. The FRMS for each concentration of GNPs (0.017±0.0001, 0.003±0.0005, 0.0004±0.00006, 0.0001±0.000009, 0±0 and 0±0 sperm/ml) and $EC_{max} = 194.98$.

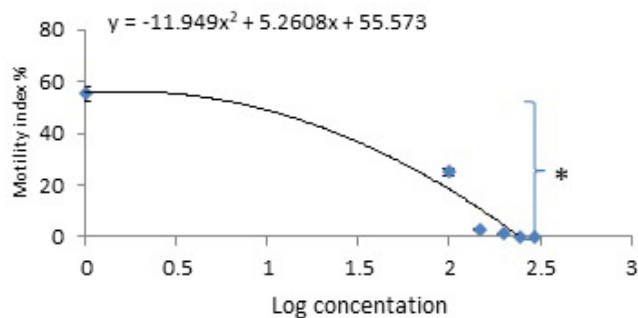


Figure 12: The Log concentrations Sperm motility index % relationship curve of mice sperm challenged Heparinized Gold Nano-particles The dated presented as SE ± Mean, the stares denoted to significant $p \leq 0.05$. n=5 mice semen suspensions The data presented as SE ± Mean, the stares denoted to significant $p \leq 0.05$. n=5 mice semen suspensions.

MOTILITY INDEX

The percentages of sperm motility index in (Figure 12) were intensively affected by GNPs and sensitive concentration changes, the control group sperm motility index and GNP-treated semen suspension were (55.46±5.82, 21.05±2.04, 8.11±1.21, 1.16±0.19, 0±0, and 0±0). The mo-

tility index was steeply decreased with an increase in GNPs at a concentration of 100 µg/ml, then fast regress with increased concentrations at 150 and 200 µg/ml significant ($p \leq 0.05$) and $EC_{50} = 19.49$, $EC_{max} = 345.8$

DNA ABNORMALITY: HISTONE RESIDUES (ALANINE BLUE STAIN)

Figure 13 investigates the concentration-dependent effects of GNPs on histone residue formation, as assessed by staining with Alanine Blue. The results demonstrate a positive correlation between GNP concentration and the observed defects. Higher GNP concentrations corresponded to a greater degree of disruption, as evidenced by the non-linear fitted line relationship. This effect was described as concentration-dependent and exhibited an aggressive deleterious impact at high concentrations. The experimental concentrations of GNPs tested were 0, 100, 150, 200, 250, and 300 µg/ml, corresponding to histone residue percentages of 1.5±0.02, 16.3±2.75, 22.33±1.42, 30.33±2.22, 34.66±4.05, and 47.33±3.81, respectively ($p > 0.05$). These findings highlight the concentration-dependent and deleterious effects of GNPs on histone residues, Figure 14. The $EC_{50} = 152.4 \pm 0.185$ µg/ml and $EC_{max} = 331.89 \pm 9.026$ µg/ml.

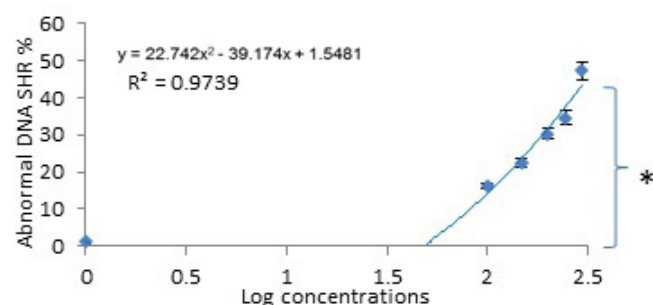


Figure 14: Concentration-response curve of the relationship between Histone Residues of sperm (Alanine Blue stain) with Log concentrations of GNPs, SHR: Sperm Histone Residue. The dated represented mean ± stander error, number of mice = 5, the star denoted significant $p \leq 0.05$.

THE INTEGRITY OF CHROMATIN AND DNA CONDENSATION (TOLUIDINE BLUE STAIN)

Figure 13 the observed result of abnormal DNA chromatin with an increased concentration of GNP, the concentrations tested of GNP were 0, 100, 150, 200, 250, and 300 µg/ml, and the result of abnormal DNA chromatin was 1.01±0.003, 29.22±0.04, 34.33±3.58, 69.32±5.21, 65.66±5.92 and 69.54±4.92), the two end concentrations displayed no significant in DNA abnormality values ($p > 0.05$), Figure 15 depicts the relationship between increasing GNP concentration (logarithmic scale) and abnormal DNA chromatin formation. The data reveal a sigmoidal dose-response curve, with a half-maximal effective concentration (EC_{50}) of 119.6 ± 0.025 µg/mL and a max imum effect (EC_{max}) of 264.8 ± 12.542 µg/ml.

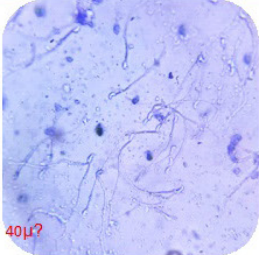
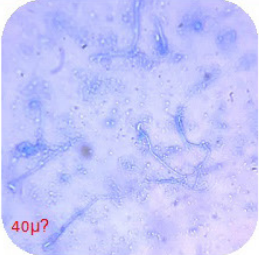
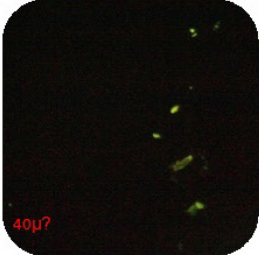
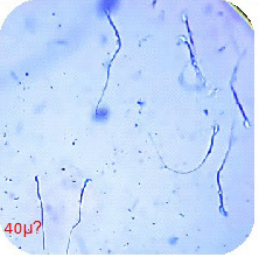
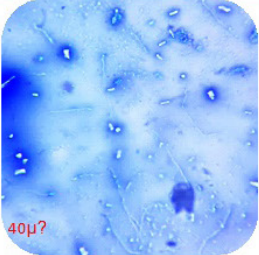
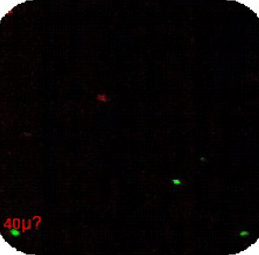
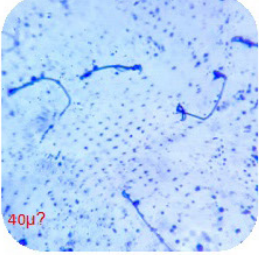
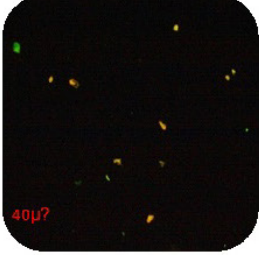
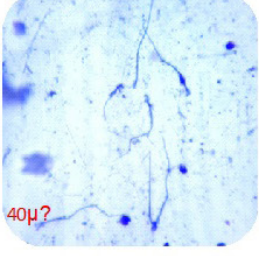
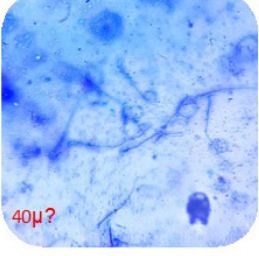
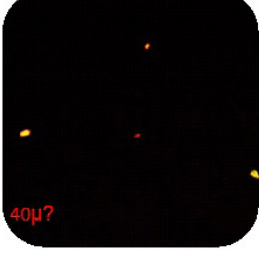
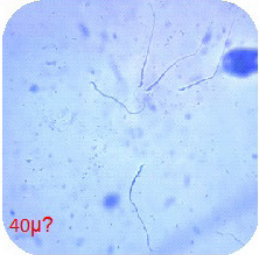
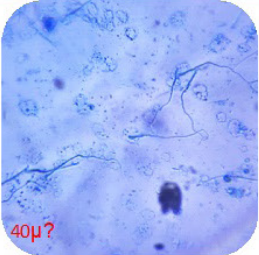
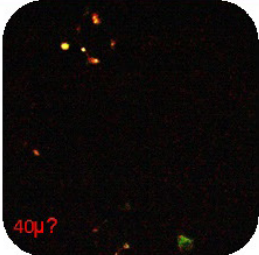
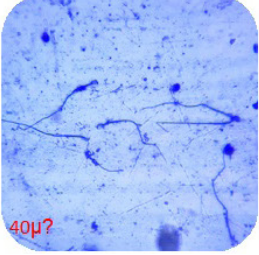
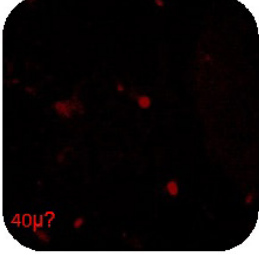
Groups	Aniline Blue	Toluidine Blue	Acridine orange
Control	 1.5±0.02	 1.01±0.003	 4.59±0.05
GNPs100µg/ml	 16.3±2.75	 29.22±0.04	 59.10±6.83
GNPs150µg/ml	 22.33±1.42	 34.33±3.58	 58.15±6.82
GNPs200µg/ml	 30.33±2.22	 69.32±5.21	 61.04±5.92
GNPs250µg/ml	 34.66±4.05	 65.66±5.92	 62.72±4.60
GNPs300µg/ml	 40.33±2.22	 79.32±5.21	 71.04±5.92

Figure 13: The DNA abnormality isolated epididymis of count sperm mice in vitro exposed to normal saline stained by Acridine orange, toluidine blue and aniline blue indicate for DNA abnormal , chromatin condensation and histone residue.

ABNORMAL SPERM DNA (ACRIDINE ORANGE)

Acridine orange staining, as illustrated in Figure 13, revealed a significant increase in abnormal sperm DNA with increasing concentrations of GNPs. The experiment employed five GNP concentrations ranging from 0 µg/mL to 300 µg/mL, while the corresponding abnormal sperm DNA levels were measured as percentages 4.59 ± 0.05 , 59.10 ± 6.83 , 58.15 ± 6.82 , 61.04 ± 5.92 , 62.72 ± 4.60 , and 60.54 ± 3.97 , respectively, the third end concentrations displayed no significant in DNA abnormality values ($p > 0.05$). The results indicate a notable relationship between the increase in abnormal DNA and the logarithmic increase in GNP concentrations Figure 16 The $EC_{50} = 13 \pm 0.025$ µg/ml and $EC_{max} = 188.36 \pm 10.638$ µg/ml.

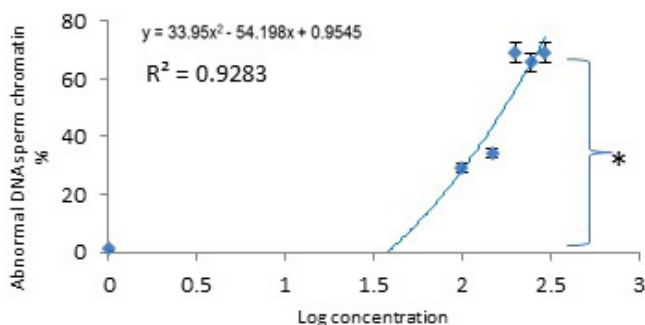


Figure 15: Concentration-response curve of Relationship between Chromatin (Toluidine Blue stain) with log concentration of GNPs. The dated represented mean $\pm$ stander error, number of mice = 5, the star denoted significant $p \leq 0.05$.

The red-brown (wine color) Gold Nanoparticle's suspension appearance was attributed to size-dependent color reported the size of Gold Nanoparticles (Gadge *et al.*, 2020; Sangwan and Seth, 2022) at less than 100 nm was given dark reddish or brown-reddish color agreed with (Vidya and Saji, 2018). The Scan EM GNP result agreed with (Oliveira *et al.*, 2023), The Scan EM exerted cubic GNPs from that was synthesized due to the method depending on polyol reduction for synthesized Cub Nano metal, as well as capping of as acidifier HCl media, which occurs the replacement of galvanic may be between heparin and $AuCl_4$ was matching this synthesized to three steps morphological structure changes cub Au first initial Au dissolution at sharp corner second bulk dissolution of Au interior through the initial site, third forming of Nano for sharp cub (Xia *et al.*, 2011). The result of XRD The outcome is attributed to the phenomenon where X-rays are specifically targeted at the atoms within the specimen, resulting in their dispersion and the creation of a diffraction pattern, which can be utilized to ascertain the crystal structure and organizational phase of the substance. The presence of peaks in an X-ray diffraction (XRD) pattern suggests the existence of a structured crystalline form in the specimen

that agrees with (Roddu *et al.*, 2020). The emergence of six distinct peaks in the XRD pattern related to Gold Nanoparticles (Anbu *et al.*, 2020).

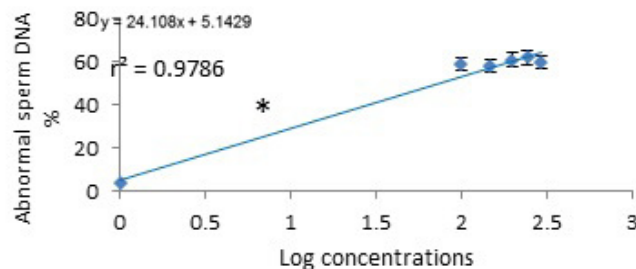


Figure 16: Concentration-response curve of Relationship between DNA abnormal (Acridine orange stain) with log concentration of GNPs. The dated represented mean $\pm$ stander error, n = 5, and the star denoted significant $p \leq 0.05$.

The quantified surface charge zeta potential provides net charges of GNP surface area which facilitates surface absorption of GNPs in an electric double layer of diffusion that develops the fact of absorption of the negative charge of zeta potential that prevents neutralization with the membrane and gives non-stability with membrane negatively charged ions. That was attributed to the accumulation of GNPs on the outer layer of the membrane without penetration in higher concentrations, which considered immobilized ions and allowed diffused according to influence opposite electrostatic force, which considered defined of electrostatic potential with a biological agent and reliable suggestion of a pinocytosis route than other routes (Wang *et al.*, 2016). The AFM showed the heterogeneity of shape GNPs with thickness range from 5.809 nm to 67.62 nm had a surface cubic to triangular Nanoparticles shape had a surface distance of 11.61 nm and vertical distance of 9 nm as well as the fraction number of size, all these were give displayed small size was highly fraction portion GNPs by heparinized methods it useful to uniform their function and that facilitated the binding of membrane and conjugated with membrane protein (Kumar *et al.*, 2008). The median lethal concentration (LC_{50}) determined by the MTT assay, which reflects sperm viability, was used to assess the lethality of GNPs in semen, the toxic mechanisms of GNP on cellular levels presumably had been elicited to adhere to cell membranes (Ghitescu and Fixman, 1984) and be endocytosis gulped by cells (Parak *et al.*, 2002). The breaching of the sperm cell membrane with the intracellular compartment storage may presumably have a dual mode negative charge and passive consequence on the sperm cells regardless of the toxic-cellularity of the GNPs and their succeeding functionality (Vijayakumar and Ganesan, 2012).

The dominance of acute toxicity escalated in the semen suspension viability exposed to treated *in vitro* with

GNP concentrations, this was particularly noticeable at the LC_{95} level, where the highest toxicity was observed. As the concentration of Nanoparticles increased, which was a corresponding gradual increase in sperm, mortality (sperm viability) was marked via MTT. This result agrees with in vitro toxicity was agreed also with MTT assay in a healthy human cell line (HaCat) and human cancer cell lines (A375, MCF-7, Bx-PC3), GNP concentrations (100, 300, and 600 μ M) resulted in a loss of cell viability (Ech-*every-Rendón et al.*, 2022; Rosłon *et al.*, 2019), may be due to the acute toxicity of GNPs on semen suspensions, which may be due to attributed to the impairment of various metabolic pathways and DNA damage (Gutiérrez *et al.*, 2017). The outcomes of the LC_{95} for sperm exposed to GNPs were attributed to the LC_{95} of GNP effect on the increase in DNA abnormalities. This is followed by defective motility, which may provide a rational explanation for the observed increase in LC_{95} concentration Figure 17, whether it be cumulative or singular causes. Furthermore, the effects presumably stem from various factors, suggesting different toxic modulated mechanisms of GNP action on semen. This initiates and progressively increases the percentage of dead sperm (Nazari *et al.*, 2016; Zakhidov *et al.*, 2010; Xiao *et al.*, 2020).

biological systems (Choi and Hu, 2008; Ringsted and Todeschini, 2012). ROS formation causes oxidative stress, inflammation, and consequent damage to the vital proteins, cell membrane, and DNA as well as advanced effects involving mitochondrial respiration and inactivation of NADPH-dependent enzyme systems (Daoud, 2007; Regoli and Giuliani, 2014; Chen *et al.*, 2011; Taha and MJ Ali, 2015). The Lag time term indicates to selection time of moving sperm to reach the light-estimated peak turbidity reflected light referring in biology to the time required from sperm deposit to migration to the fertilizing area (Sokoloski *et al.*, 1977). The period depends on the velocity of sperm rapidly moving sperm and a detectable number of motile speedy sperm. The delay of Lag time as concentration-dependent was presumably attributed to the reduction of sperm velocity (Sokoloski *et al.*, 1977).

The velocity of the fraction of rapidly moving sperm depends on several vital processes due to the direct effect GNPs on sperm structure or metabolic direct or indirect by deprived ATP demined may be through decrease eventually impaired metabolic pathway (Li *et al.*, 2023). The reduction in VRMS presumably directly causes Loss of fluidity and elasticity of the sperm's outer structure, disposal of the tail axial fiber structure of kinetic motion of sperm, and loss of partial regular vigorous forward movement. As well as the GNP reduced activity of GNP on the sperm tail limiting mitochondria directly through translocation transport and Plasmon biocompatibility. That presumably led to reduced metabolic activity reduction of ATP demined (Xiao *et al.*, 2020).

The fact of indirect defective mechanisms affected by GNP including free radical dominance may be due to i. Oxidant-generating properties of particles themselves as well as their ability to stimulate the generation of ROS as a part of cellular response to Nanoparticles ii. Transition metal-based Nanoparticles or transition metal contaminants are used as catalysts during the production of non-metal Nanoparticles. iii. Relatively stable free radical intermediates are present on reactive surfaces of particles. iv. Redox-active groups result from the functionalization of nanoparticles (Alani *et al.*, 2011; Fard *et al.*, 2015). The FRMS may be affected due to the direct effect of GNP on spermatozoa membrane and their structures suggesting the importance of structural membranous ionic regulatory action potential and their control of Ca^{2+} channels, which is essential for sperm motility. The Ca^{2+} permeation pathway in sperm motility is essential for mammalian fertilization, their report attributed a candidate sperm cation channel, Cat Sper, that has an amino-acid sequence similar to a single. Sperm motility is significantly reduced in CatSper blocker in mice. Moreover, the cAMP-induced Ca^{2+} influx is eliminated in the sperm of blocked this channel. Therefore, Cat Sper is crucial for cAMP-mediated Ca^{2+} influx in

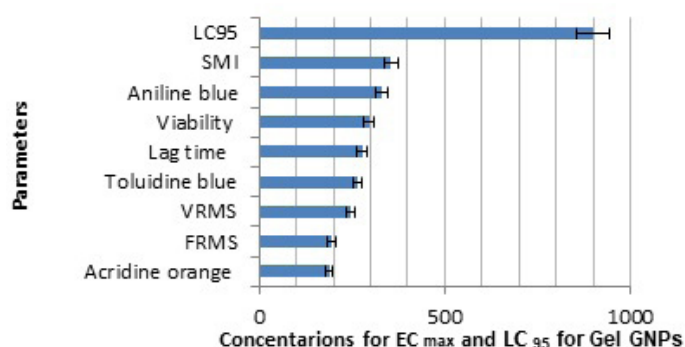


Figure 17: The potency of sperm-toxic GNP effect represented by the A. LC_{95} value.

The internalization of GNPs within cells can potentially alter their stability and permeability due to interactions with the cellular environment, including the cell membrane. Cationic nanoparticles, with their positive charge, tend to exhibit a higher affinity for negatively charged cell membranes, facilitating their uptake even by non-targeted cells (Ferreira-Gonçalves *et al.*, 2021; Singh *et al.*, 2019). The Cage rectangular, hexagonal, and prism forms of GNPs result in a differentiated distribution of Gold atoms on to the cellular surface, with more edged and angular Nanoparticles endorsing greater reactivity, but it differs the spherical form being more efficiently internalized by cells (Kus-Liskiewicz *et al.*, 2021; Jia *et al.*, 2017; Behzadi *et al.*, 2017; Didamson *et al.*, 2022). The concept of the surface area of GNPs is an essential element as Pro-oxidants in their tense toxicity due to their interaction with

sperm, and sperm motility (Ren *et al.*, 2001).

The findings of SMI results were consistent with those of (Wiwanitkit *et al.*, 2009) observed that Gold Nanoparticles exerted dose-dependent effects on sperm motility in their study on donor sperm. Similarly, found that both silver and Gold Nanoparticles influenced sperm motility in a dose-dependent manner (Moretti *et al.*, 2013). It is agreed to highlight that research focusing on the impact of gold nanoparticles on the male reproductive system is scarce. The result of sperm motility index at exposure to GNP concentrations decreased sperm motility this result agrees with (Nazari *et al.*, 2016). While reaching the end two concentrations of Gold Nanoparticles (250, 300 µg/ml) presented absent sperm motility and attributed the effect of GNPs on the motility of sperm to numerous causes, including defect of cell membrane integrity. That led to decreased fluidity and elasticity, as well as the presumably causal of GNP exerted via the GNP have high affinity to free thiol on sperm surface aggregation that causes a massive loss of motility. The thiol is a component of membrane-bound Na⁺/K⁺-ATPase and Ca²⁺-ATPase, which were major responsible for the initiation and demined of sperm motility (Hanon *et al.*, 2022; Taylor *et al.*, 2014).

An increased concentration of Gold Nanoparticles led to a significantly increased ($p \leq 0.05$) abnormal percentage of Histone residues as compared with the group of control semen suspension. The GNP-treated semen suspension groups increased the histone defect as concentrations dependent on these results agreed with (Nazari *et al.*, 2016). The protamine-like proteins that under normal conditions played an important role in the condensation and stabilization of gamete's nuclear chromatin. The results of toluidine blue were denoted to abnormal chromatin and referred to the decondensation pattern of the nucleosome, which means a reduced number of protamines, resulting in an effect on sperm genetic sources (Zakhidov *et al.*, 2010). In addition, the rate of abnormal chromatin condensation was increased when exposure sperm was to high concentrations of Gold Nanoparticles significantly ($p \leq 0.05$) at the two-end concentration of Gold Nanoparticles (250 and 300 µg/ml) displayed no significance in DNA abnormality value ($p > 0.05$) (Zakhidov *et al.*, 2010). The results noticed in this outcome of stain were a positive correlation with an increased Gold nanoparticle concentrations significantly ($p \leq 0.05$) reflex to the increased rate of abnormal DNA. That includes DNA fragmentation, and chromatin decondensation due to maybe decreased protamine confirmation and turnover and increased rate of histone residue, these results of presumably the DNA, GNPs can directly interact with DNA and cause DNA fragmentation. That is the breaking of DNA strands into smaller pieces GNPs can induce chromatin decondensation, it is loose of the tightly packed DNA-protein complex that forms the chro-

mosomes. This can result from the alteration of protamine and histone proteins, which are responsible for maintaining the stability and compaction of chromatin (Nazari *et al.*, 2016; Wang *et al.*, 2017).

CONCLUSIONS AND RECOMMENDATIONS

The conclusion The study successfully prepared heparinized Gold nanoparticles (GNP) and tested them in both in vitro and in vivo experiments. These GNPs, synthesized particularly in an acidic environment with heparin, formed a cubic-cubic cage shape and were less than 100 nm in size. They exhibited stability, crystalline properties, and homogeneous surface roughness. In the in vitro experiment, the GNPs demonstrated spermicidal efficacy of more than 300 µg/ml. They affected sperm motility and limited the proportion of rapidly moving sperm at concentrations less than LC values. The GNPs also induced DNA abnormalities with higher efficacy than other vital functional motivations. These findings suggest that the GNPs, when used in Gel GNP vaginal spermicides, could minimize vaginal pathological deterioration, making them a potential candidate for contraception.

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NOVELTY STATEMENT

The novelty of the research is focused on the toxicity of gold nanoparticles leading to induced the toxicity effects on sperm mice in vitro.

AUTHOR'S CONTRIBUTION

These researchers each contributed equally to this research.

CONFLICT OF INTEREST

There is no conflict of interest.

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