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ASSESSMENT OF THE INFLUENCE OF SILVER IONS AND SILVER NANO-PARTICLES ON THE REPRODUCTIVE STATE, LIVER FUNCTION AND RENAL FUNCTION OF ADULT WISTAR RATS

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ABSTRACT

Background of Study: The pharmacological benefits of ionic and nano particles have been widely speculated and reported, but data about their effects on fertility is grossly scarce. This research was however, carried out to study how these particles influence fertility, hepatic and the renal functions. Materials and methods: Sixty four (64) adult Wistar rats were divided into nine (9) groups, viz, NO, LD (nano particles, oral administration at low dose), NO, HD (nano particles oral administration at high dose), NP, LD (nano particles intraperitoneal administration low dose, NP, HD (nano particles intraperitoneal administration high dose), I+NO LD (ionic silver and Nano-particles, oral administration low dose, I+NO HD (ionic silver and Nano-particles, oral administration high dose, I+NP LD (ionic silver and nano-particles, intraperitoneal administration low dose), and I+NP HD - Ionic silver and nano-particles, intraperitoneal administration high dose). **Results:** The groups treated with NO, LD and NO, HD demonstrated greater potential benefits in positively enhancing fertility in the treated female and male rats. The NO, LD group had the highest plasma level of LH (4.47 mul/mL) compared to the control (1.37 mul/mL) and the other groups, as well as significantly decreased plasma testosterone concentration (0.00 ng/mL) compared to the control group (0.10 ng/mL) and the other groups. The NO, LD group also demonstrated a significantly high plasma FSH concentration (5.70 mul/mL) and plasma estrogen concentration (110.53 pg/mL). FSH (and LH) stimulates testicular growth and enhances the production and maturation of sperm cells. LH (25.65 mul/mL) and FSH (32.75 mul/mL) increased significantly compared to the control group, LH (5.55 mul/mL) and FSH (4.85 mul/mL), also suggesting that low dose of the particles better enhanced fertility of the treated male group. **Conclusion:** From the results, oral administration of low dose nano-particles could enhance the fertility of male and female

KEYWORDS: Ionic Silver, Nano-particles, Sperm, Liver, Wistar rat.

INTRODUCTION

The pharmacological benefits of silver ions (from dissolved silver nitrate and silver sulfadiazine) have been known for ages, especially its antibacterial properties[1], but with the advent of nanotechnology, it was remodified into nanoparticles (AgNPs), with highly developed surface area to size ratio. The potentials of silver ions and AgNPs against infections been grossly reported. Silver ions have been reported to be highly reactive, interacting with molecules (chloride ions) and biomolecules (albumin) to form insoluble compounds. AgNPs occurs in different shapes depending on the mode of production, e.g., using biological systems for its synthesis (bacterial, fungi, plants, etc.). AgNPs have the potential to form aggregations so that it requires carriers and stabilizers like organic (e.g., citrate) and inorganic (e.g., silica, graphene, titanium, etc.) for their bioadministration. Silver ions have been reported to more active than AgNPs[2], as it is able to interact with structural and functional proteins, especially those with thiol functional groups (-SH)[3], and respiratory chain proteins, especially in microbes. The cytotoxicity of these particles on osteoblasts and osteoclasts have been studied and reported[4], in which there were severe alterations of cellular differentiation and viability.

Information about the effects of these particles on reproduction, hepatic and the renal functions are scarce, thus, this research was designed to elaborate on this premise, in which we assessed the influence of silver ions and silver nano-particles on the reproductive state, liver function and renal function of adult Wistar rats.

MATERIALS AND METHODS

Rats used for the Study

Sixty four (64) male and female adult Wistar rats were housed in metal cages in the animal house at ambient temperature and allowed to acclimatize for one week before admiration begun.

Sperm Cell Collection

The sperm cells were isolated from the vas deferens of the sacrificed rats the following standard methods.

Sperm Cell Analysis

Spermatozoa motility, vitality testing, morphology assessments and sperm cells count were carried out using standard laboratory techniques.[5,6,7,8,9,10,11,12]

Biochemical and Hormonal Assays

The hormones, hepatic and renal assays were done using standard biochemical procedures and kits.

Silver Particles Used

Silver particles (average sizes of 50 nm, 3 µm, 30 µm, 8 µg/ml – 500 µg/ml) and Ag+NO3- (0.5 µg/ml – 500 µg/ml) of Sigma Aldrich co., Germany origin were obtained prior to the treatment and analysis. Silver treatment started on day 3 to prevent interference with cell adhesion.

Histological Analysis

Processing of tissues (Reproductive, liver and Renal tissues) using routine biopsy method was employed. Tissue sections were treated with traditional haematoxylin and eosin stains. Tissue blocks from the heart and blood vessels were fixed in 10% neutral formalin after which they were dehydrated using alcohol and cleaned in xylene. The samples were embedded in paraffin wax and thin sections cut at 5 microns. The sections were then stained with haematoxylin for 15 minutes, differentiated with 1% acid alcohol, counter stained in eosin for 2 mins and mounted with DPX. The sections were viewed under the microscope at x400 magnification and photomicrographs taken.

Doses of the Particles Used For the Study

Ionic silver was administered to the rats at a dose of 0.036mg/kg body weight.

Nanoparticles was administered to the animals at a dose of 0.112mg/kg body weight.

Protocol of Treatment

C – Control
NO, LD - Nano particles, oral administration at low dose
NO, HD - Nano particles oral administration at high dose
NP, LD - Nano particles intraperitoneal administration low dose
NP, HD - Nano particles intraperitoneal administration high dose
I+NO LD - Ionic silver and Nano-particles, oral administration low dose
I+NO HD - Ionic silver and Nano-particles, oral administration high dose
I+NP LD - Ionic silver and Nano-particles, intraperitoneal administration low dose
I+NP HD - Ionic silver and Nano-particles, intraperitoneal administration high dose

Statistical Analysis

The data were entered into Microsoft Excel Spread sheet prior to analysis, and the analysed using the SPSS one-way Duncan's multiple range analysis of variance. The p-values greater than 0.05 were considered to be significant.

RESULTS

Table 1: Sperm cell analysis of the low dose and high dose ionic silver treated and control cells.

Study group	Sperm cell Aggregation			Sperm cell morphology		Sperm cell motility			
	Head to head	Body to body	Tail to tail	Normal (%)	Abnormal (%)	PM (%)	NPM (%)	IM (%)	TSC (x10^{6}cells/mm^3)
Control	-	-	-	80.00±0.00	8.00±0.00	85.00±5.00	5.00±0.50	10.00±0.00	305.00±10.00
Ionic LD	38.00±9.00	12.00±5.00	20.00±4.00	74.00±2.00	26.00±2.00	66.00±2.00	16.00±4.00	16.00±2.00	215.00±10.00
Ionic HD	13.33±0.60	13.33±0.60	10.00±0.10	73.33±0.30	26.67±0.30	60.00±1.00	13.33±0.30	26.67±0.80	243.33±3.00
F value	18.406	1.733	13.920	36.541	41.866	27.225	40.751	39.055	39.256
p-value	0.000	0.163	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Percentage Sperm cell motility, count and quality and percentage sperm cell morphology (abnormalities and normal phenotypic appearance) across the study group (Ionic) and Control group. **PM**=Progressive motility, **NPM**=Non Progressive Motility, I**M**= immobility, **TSC**=Total Sperm cell count. **TSC**= X10^{3}cells/mm^3. Normal (matured sperm cell with full anatomic features, morphology; head; body, axonemal and tail). Abnormal (immature spermatozoa, degenerated sperm cell debris, headless/ tailless, bulgy neck, bloated head, short sperm cells).

Table 2: Sperm cell analysis of the low dose and high dose Nano-particle treated and control cells.

Study group	Sperm cell Aggregation			Sperm cell morphology		Sperm cell motility			
	Head to head	Body to body	Tail to tail	Normal (%)	Abnormal (%)	PM (%)	NPM (%)	IM (%)	TSC (x10^{6}cells/mm^3)
Control	-	5.00±0.50	-	91.25±1.00	8.75±0.10	85.00±0.30	5.00±0.30	10.00±0.00	305.00±2.00
Nano-particle LD	26.00±1.00	6.00±0.40	2.00±0.20	76.00±0.20	24.00±0.20	56.00±0.60	20.00±0.40	26.00±0.90	286.00±1.00
Nano-particle HD	12.00±1.00	14.00±0.40	8.00±0.50	80.00±0.00	20.00±0.00	78.00±0.20	10.00±0.00	12.00±0.20	298.00±1.00
F value	27.307	44.110	31.663	37.229	45.062	36.008	53.111	22,055	11.530
p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Percentage Sperm cell motility, count and quality and percentage sperm cell morphology (abnormalities and normal phenotypic appearance) across the study group (Ionic) and Control group. **PM**=Progressive motility, **NPM**=Non Progressive Motility, I**M**= immobility, **TSC**=Total Sperm cell count. **TSC**= X10^3cells/mm^3. Aggregation study (head to head, body to body and tail to tail). Abnormal (immature spermatozoa, degenerated sperm cell debris, headless/ tailless, bulgy neck, bloated head, short sperm cells).

Table 3: Hormonal profile of the female treated rats.

Tests	LH (mul/mL)	FSH (mul/mL)	Prolactin (ng/mL)	Testosterone (ng/mL)	Progesterone (ng/mL)	Estrogen (pg/mL)
Control	1.03±0.60	1.37±0.90	1.50±1.00	0.10±0.00	3.30±0.30	99.37±5.00
NO, LD	4.47±0.30	5.70±0.20	2.23±0.20	0.00±0.00	3.57±0.60	110.53±9.00
NO, HD	2.77±0.50	2.70±0.70	3.23±1.00	0.67±0.00	4.30±0.50	119.00±9.00
NP, LD	1.10±0.60	4.90±0.20	3.10±0.10	0.37±0.00	4.17±0.04	109.33±4.00
NP, HD	1.37±0.40	1.80±0.00	3.73±0.60	0.23±0.00	2.60±0.50	95.80±5.00
I+NO, LD	1.60±0.80	2.07±0.10	3.77±0.20	0.23±0.01	3.20±0.80	106.50±16.00
I+NO, HD	0.60±0.00	1.07±0.20	2.00±0.01	0.23±0.00	2.20±0.10	128.50±2.00
I+NP, LD	0.00±0.00	1.93±0.50	2.07±0.20	3.83±0.30	0.37±0.01	2.23±0.20
I+NP, HD	1.60±0.50	6.83±0.50	12.33±0.80	0.20±0.01	2.97±0.50	107.00±4.00
F value	39.520	42.711	45.066	35.228	38.640	81.502
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Table 4: Hormonal profile of the male treated rats.

Tests	LH (mul/mL)	FSH (mul/mL)	Prolactin (ng/mL)	Testosterone (ng/mL)	Progesterone (ng/mL)	Estrogen (pg/mL)
Control	5.55±0.20	4.85±0.20	2.35±0.20	1.35±0.10	3.75±0.03	121.00±0.00
Ionic low dose	25.65±0.40	32.75±0.30	8.05±0.06	1.00±0.01	3.50±0.06	114.00±0.06
Ionic high dose	2.83±0.01	4.33±0.02	1.87±0.01	0.80±0.06	2.77±0.05	125.53±020
F value	33.071	37.291	20.643	7.551	6.413	11.805
p-value	0.000	0.000	0.003	0.043	0.031	0.000

Table 5: Liver and renal profile of the male and female treated rats.

	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	TBIL (mg/dl)	DBIL (mg/dl)	Na^+(mEq/L)	K^+(mEq/L)	Cl^-(mEq/L)	UREA (mg/dl)	CREAT. (mg/dl)
Control	10.32±0.90	19.45±0.90	118.60±24.00	7.65±0.80	0.17±0.03	0.12±0.03	146.60±6.00	8.40±2.00	125.40±29.00	19.15±3.00	2.41±0.60
NM	10.80±0.40	20.00±1.00	136.80±30.00	6.09±2.00	0.09±0.02	0.05±0.02	167.80±5.00	5.29±0.60	112.40±16.00	27.66±6.00	12.02±4.00
NF	10.06±0.20	18.33±1.00	130.14±30.00	7.38±2.00	0.10±0.40	0.04±0.05	162.37±3.00	5.40±0.20	111.90±11.00	25.26±2.00	11.93±2.00
p-value	0.071	0.039	0.004	0.018	0.042	0.040	0.015	0.046	0.039	0.006	0.038
IM	10.20±0.90	18.00±4.00	238.60±48.00	6.49±2.00	0.12±0.03	0.15±0.60	149.00±7.00	7.48±0.80	44.60±13.00	32.82±3.00	5.68±2.00
IF	9.11±0.60	16.30±1.00	231.09±32.00	6.65±1.00	0.17±0.07	0.11±0.10	134.85±3.00	7.76±0.40	44.02±11.00	31.00±4.00	5.30±3.00
p-value	0.056	0.021	0.011	0.035	0.000	0.000	0.000	0.000	0.042	0.045	0.039

C=control; N=nano particle; I=ionic particle; M=male; F=female.

Table 6: Antioxidant profile and lipid peroxidation marker level of the female low dose treated rats.

	MDA (×10^{-3} mmole/mL)	GPx (U/mL)	SOD (U/mL)	CAT (U/mL)
Control	2.20±0.80	91.01±34.00	22.847.00	9.18±0.50
NO LD	4.09±3.00	87.10±21.00	32.1967±8.00	9.9400±0.60
NO HD	1.98±0.07	45.45±1.00	35.12±7.00	11.45±0.80
p-value	0.000	0.000	0.006	0.000
I+NO LD	1.59±0.50	77.08±11.00	52.08±4.00	6.97±1.00
I+NO HD	1.77±0.40	68.84±11.00	45.60±3.00	10.67±2.00
p-value	0.002	0.000	0.000	0.000
NP LD	1.41±0.10	89.61±24.00	18.99±10.00	10.44±0.90
NP HD	2.34±1.00	76.77±10.00	43.50±5.00	8.38±1.00
p-value	0.004	0.000	0.000	0.000
1+NP LD	1.86±0.30	119.49±10.00	41.86±9.00	9.48±10.00
1+NP1 HD	2.72±0.70	73.85±15.00	41.29±0.80	8.81±0.40
p-value	0.000	0.000	0.098	0.062

Table 7: Antioxidant profile and lipid peroxidation marker level of the male low dose treated rats.

	MDA ($\times 10^{-3}$ mmole/mL)	GPx (U/mL)	SOD (U/mL)	CAT (U/mL)
Control	9.14±0.20	387.55±64.00	96.62±0.005	3.23±0.30
NO LD	5.20±1.00	83.33±17.00	34.14±3.00	7.27±0.30
NO HD	2.11±0.02	46.05±0.80	37.08±5.00	13.55±0.60
p-value	0.000	0.000	0.000	0.000
I+NO LD	1.61±0.08	78.11±11.00	57.11±0.40	7.16±6.00
I+NO HD	1.79±0.20	63.05±11.00	44.25±0.20	11.04±5.00
p-value	0.000	0.000	0.000	0.000
NP LD	1.66±0.30	90.25±19.00	17.53±6.00	14.32±0.60
NP HD	2.50±0.20	78.33±14.00	45.22±2.00	11.05±0.40
p-value	0.000	0.000	0.000	0.000
1+NP LD	1.37±0.10	125.30±9.00	53.27±0.30	9.30±0.60
1+NP1 HD	2.55±0.20	78.41±10.00	46.60±0.80	11.05±0.20
p-value	0.000	0.000	0.000	0.000

Commentary on Histology

Testis

The microstructure of the testis in the control group shows normal features composed of Semiferous tubules lined by spermatocytes in normal sequential maturation, supported by sustenocular cells of sertili: the cut section of tubules also shows the interstitial space, containing Leyclig cells and pierced by branches of the testicular artery. Treatment of the Wistar rats with low dose nano silver particles showed focal spermatogenic arrest and active interstitial congestion and vasodilatation in the testis. The structure and morphology of the Sertoli and Leydig cells appeared normal. Whereas low dose Ionic Silver particles induced mild interstitial oedermol, degeneration of the spermatocytes and patchy spermatogenic arrest.

Epididymis

The morphology of the epididymis in the control group revealed normal features composed of ducts (tubules) separated by interstitial space and packed with mature spermatozoa. On treatment with low dose silver nano particles there was mild to moderate depletion of luminal spermatozoa. When treated with low dose Ionic Silver particles there was mild to moderate depletion of spermatozoa in some ducts and severe depletion of spermatozoa in others.

Ovary

The microstructure of the rot ovary in the control group show normal features composed of graffian follicle, ovum and stromol. Treatment with low dose Silver Nano particles showed follicles in different stages of development and leutenized stromol as well as moderate stromol congestion. High dose silver nano particles induced degeneration of follicles as well as inhibiting follicular maturation. There was sever stromol congestion. Treatment with low dose ionic Silver particles induced severe stro mal congestion and severe stromal leutenization. The follicles are at different stages of development with increased folliculogenesis. High close ionic silver particles included severe stromal congestion, severe stromal leutenization and increased folliculogenesis.

Uterus

The histo-architecture of the control group roots showed normal morphology composed of endometrial cavity and lining, endometrial stromal, endometrial glands and myometrium. Treatment with low dose silver nano particles induced moderate stromol congestion and oedemol as well as moderate infiltrate of stromol inflammatory cells. High dose silver nano particles induced glandular epitheliosis, moderate stromal congestion, oedemol and infiltrates of inflammatory cells. Treatment with low dose ionic silver particles induced mild endometrial lining and glandular epitheliosis as well as hyperplesia. High dose ionic silver particles induced moderate degrees of hyperplesia of the endometrial lining, stromol and glands.

Brain

The histo-morphology of the brain shows normal features composed of the molecular layer, branches of the cerebral vessels and arachnoid granulations. Treatment with low dose silver Nano shows moderate vascular congestion, ulceration and stenosis. High dose silver nano particles induced severe vascular congestion and ulceration. Treatment with low dose ionic silver particles induced severe vascular congestion and ulceration. High dose ionic silver particles induced severe vascular congestion and vascular distention.

Histology key: Abnormal= Headless, Tailless, Bulgy midpiece, short tail, bent head, multiple head Normal= Head, Midpiece (Axonem) Tail all in Proportion

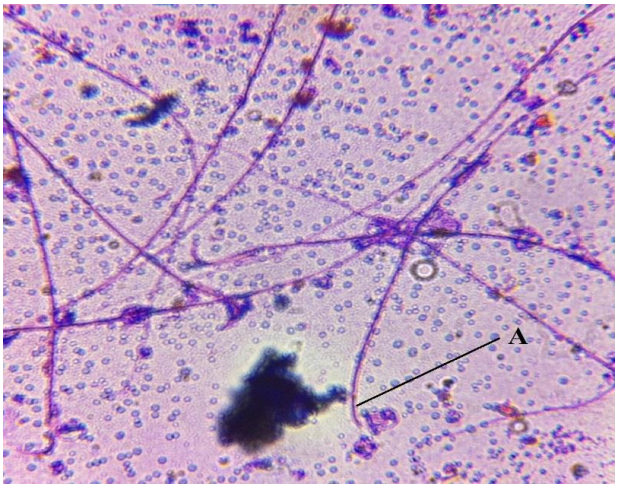

Figure 1: (Ionic 1) (A) abnormal spermatozoa, Headless sperm cell.

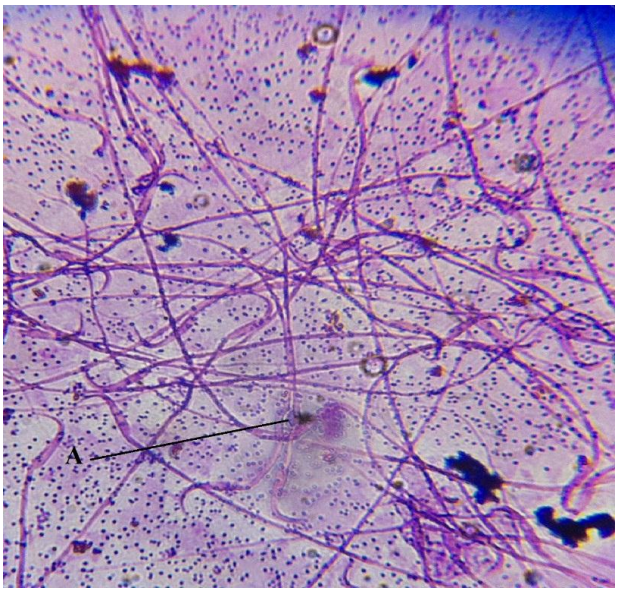

Figure 2: (Ionic 2) (A) Abnormal Sperm cell, Bulgy midpiece.

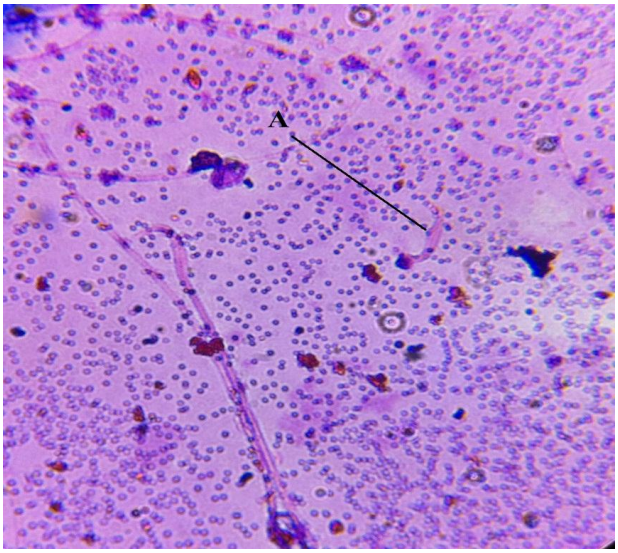

Figure 3: (Ionic 3) (A) Tailless spermatozoa (abnormal).

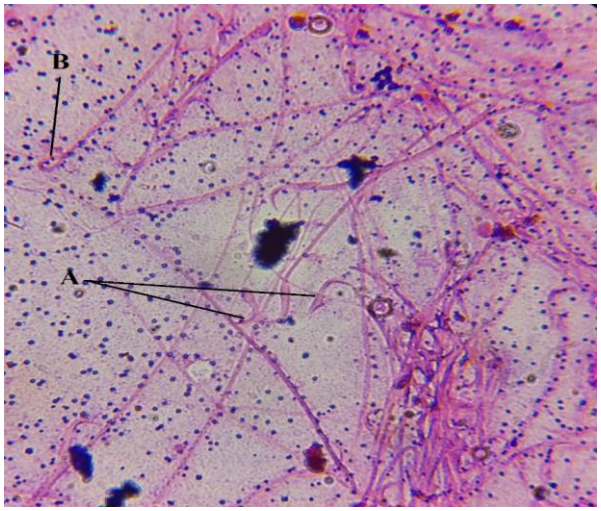

Figure 4: (A) Headless spermatozoa (B) bent headed spermatozoa (abnormalities).

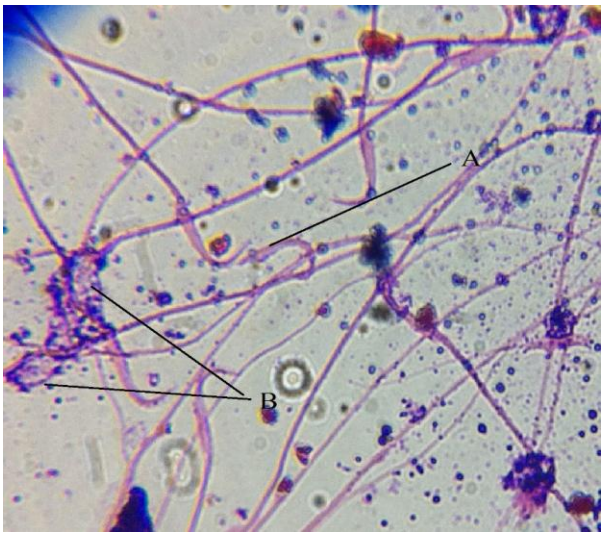

Figure 5: (A) Tailless (B) Degenerated Spermatozoa (abnormalities).

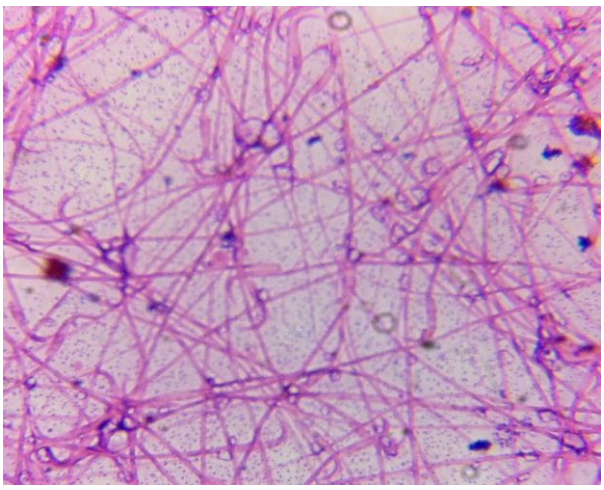

Figure 6: (Control 1) Numerous sperm cells, normal count, normal Spermatozoa.

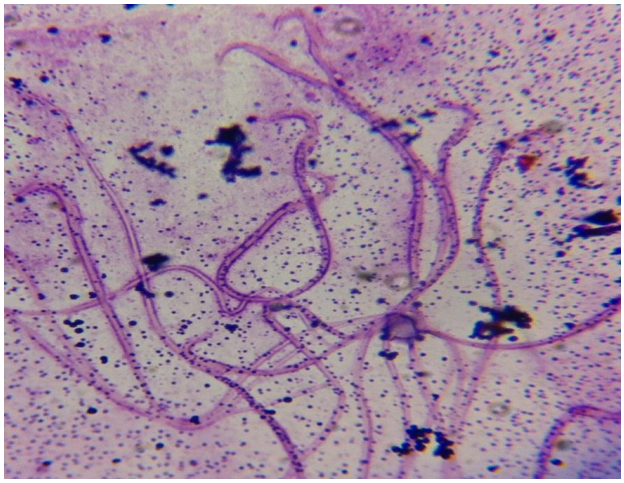

Figure 7: (Control 2).

DISCUSSION

In this research, experiments to the ascertain the effects of ionic silver and nano-particles on reproduction, hepatic and the renal functions of adult Wistar rats were carried out, compared to the control group. Specifically, the influence of ionic silver and nano-particles on the morphology of sperm cells and hormonal profile where ascertained. From Table 1, the male groups given the ionic silver particles (low and high dose) showed significant increases in head to head, body to body and tail to tail aggregations, compared to the control group. There was a significantly increased percentage number of abnormal sperm cells with dose, which also corroborates with increased progressive motility, non progressive motility and immobility. There was also a significant decrease in total sperm cell count with the low dose, with an increase in the high dose but not close to the control group. Table 2 which represented the groups administered with the nano-particles also demonstrated that there were significant increases in the head to head, body to body and tail to tail aggregations of the sperm cells, especially with an increase in dose of the administered nano-particles (but not with the head to head in which there was a decrease in the high dose). The percentage abnormal sperm cell and non progressive motility increased with the dose of administered nano-particles, with the progressive motility of the cells showing inconsistent variations of decrease at low dose and then increase with the high dose.

The hormonal profile after treatment is represented in Table 3 and 4. The treated female rats (Table 3) showed that the NO, LD group had the highest plasma level of LH concentration (4.47 mul/mL) compared to the control (1.37 mul/mL) and the other groups, as well as significantly decreased plasma testosterone concentration (0.00 ng/mL) compared to the control group (0.10 ng/mL) and the other groups. The NO, LD group also demonstrated a significantly high plasma FSH concentration (5.70 mul/mL) and plasma estrogen concentration (110.53 pg/mL). FSH (and LH) stimulates testicular growth and enhances the production and maturation of sperm cells. In females, LH (and FSH) controls the menstrual cycle and triggers the release of an egg from the ovary (ovulation), so that, LH increases during ovulation, thus, promoting fertility (in both males and females). Decreased LH and FSH levels can cause infertility, menstrual difficulties, low sex drive and delayed puberty. It is also important to mention that LH helps the testicles to produce testosterone, which is important in sperm cell production. However, the I+NP, HD group had the highest plasma FSH concentration (6.83 mul/mL) and plasma prolactin (12.33 ng/mL). The I+NP, LD group demonstrated a significantly increased plasma testosterone concentration (3.83 ng/mL) compared to the control (0.10 ng/mL) and the other groups. This increase in testosterone is followed by a significantly decreased plasma LH (0.00 mul/mL). However, the I+NO, HD group had the highest plasma estrogen concentration (128.50 pg/mL), followed by the NO, HD group (119.00 ng/mL, with plasma progesterone level of 4.30 ng/mL), compared to the control group (99.37 pg/mL). After ovulation, as regulated and promoted by LH and FSH, the ovaries are further stimulated by these hormones to produce estrogen and progesterone (i.e., post-ovulation). Estrogen and progesterone stimulate the uterus and breast to prepare for possible fertilization and implantation. Conversely, the group treated with I+NP, LD demonstrated a reverse profile against the NO, LD group.

Thus, in females, estrogen peaks pre-ovulation (follicular phase) persisting into the ovulation proper; FSH and LH peaks at ovulation, triggering the release of an egg from the ovary (ovulatory phase). These events re followed by surge of progesterone from the *corpus luteaum* (luteal phase), with further persistence of estrogen to prepare the uterus to house an embryo, if fertilization and implantation occurs.

As have been observed, the three phases of the menstrual cycle were visibly stimulated (the follicular, ovulatory and luteal) in the groups treated with NO, LD and NO, HD, suggesting their potential benefits in positively enhancing fertility in the treated female rats. These observations also corroborates with the male group treated with ionic low dose (Table 4) in which LH (25.65 mul/mL) and FSH (32.75 mul/mL) increased significantly compared to the control group, LH (5.55 mul/mL) and FSH (4.85 mul/mL), also suggesting that low dose of the particles better enhanced fertility of the treated male group.

The influence of ionic and nano particles where also studied and represented in Table 5. The plasma ALT of the treated groups decreased compared to the control (10.32 U/L) except the male group treated with nano-particles (NM; 10.80 U/L). Plasma AST followed similar fashion ALT, whereas, plasma ALP, urea and creatinine for all the treated groups increased significantly compared to the control group, while plasma GGT for all the female group decreased. The data from Table 6 (female) and 7 (male) showed that plasma MDA

concentration of the NO LD group increased markedly compared to control and the other groups that varied slightly. The patterns of the plasma antioxidant activities showed significant incremental variations for the groups compared to the control.

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