Modern Research in Inflammation, 2024, 13, 29-41
https://www.scirp.org/journal/mri
ISSN Online: 2169-9690
ISSN Print: 2169-9682

# Caffeine Intervention Ameliorates Effect of Nigerian Bonnylight Crude Oil on Sperm Motility and Morphology of Diabetic Wistar Rats

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**How to cite this paper:** Nwariri, E., Ita, S.O., Udokang, N.E., Abia, E.R. and Umoh, C.N. (2024) Caffeine Intervention Ameliorates Effect of Nigerian Bonnylight Crude Oil on Sperm Motility and Morphology of Diabetic Wistar Rats. *Modern Research in Inflammation*, **13**, 29-41.
https://doi.org/10.4236/mri.2024.133003

**Received:** June 30, 2024
**Accepted:** August 27, 2024
**Published:** August 30, 2024





## Abstract

The study investigated the intervention of caffeine on the effect of Nigerian Bonnylight crude oil (NBLCO) on sperm motility and morphology of diabetic Wistar rats. Eighty adult male rats (180 - 200 g body weight) were randomly divided into eight groups of 10 animals each. The control group received 3 mL/Kg body weight of distilled water, ND Caf. group received 20 mL/kg body weight of caffeine, NDCO group received 3 mL/Kg body weight of NBLCO, ND Caf. + CO group received 20 and 3 mL/Kg body weight of caffeine and NBLCO respectively, diabetic group received 3 mL/Kg body weight of distilled water, D Caf. group received 20 mL/Kg body weight of caffeine, D CO received 3 mL/Kg body weight of NBLCO and D Caf. + CO group received 20 and 3 mL/Kg body weight of caffeine and NBLCO respectively, by oral gavaging for 28 days. The results showed that independent administration of caffeine and NBLCO to both non-diabetic and diabetic rats significantly ($p < 0.05$) altered sperm concentration, morphology and motility. Administration of NBLCO aggravated the worsening condition by significantly ($p < 0.05$) reducing sperm concentration, motility and increasing abnormal sperms in diabetic rats. Although caffeine significantly ($p < 0.05$) reduced motility in non-diabetic rats, it did cause any significant alteration in diabetic rats. Diabetes mellitus and NBLCO significantly ($p < 0.05$) reduced all indices that promote sperm motility while significantly ($p < 0.05$) increasing indices that do not promote motility. It can be concluded that diabetes mellitus and NBLCO administration have shown the tendency to promote male infertility by reducing sperm motility and adversely altering the morphology of sperm in male Wistar rats, which was ameliorated by caffeine intervention.

**Keywords**

Caffeine, Nigerian Bonnylight Crude Oil, Sperm Motility, Sperm Morphology, Male Infertility

## 1. Introduction

The continuous contamination of the ecosystem by xenobiotic agents like the Nigerian Bonnylight crude oil has demonstrated both direct and indirect adverse impacts on human and other animals' health in diverse ways, especially in the Niger Delta region of Nigeria. This is particularly due primarily to the presence of polycyclic aromatic hydrocarbons (PAHs), as well as other toxic constituents [1] [2]. There can be various pathological conditions affecting the bodily systems, organs and tissues associated with blood and immune system, as well as neurodegenerative disorders in the nervous system, pulmonary system, cardiovascular system, reproductive and endocrine systems, renal, and hepatic diseases, arising from unrestricted exposure. These xenobiotic agents seem to exert their hazardous effect by the generation of excessive oxidant radicals, which impose a huge burden on the endogenous antioxidant systems, resulting in oxidative stress. The oxidative stress development heralds the cascade of activities leading to peroxidation of lipids, inflammation, altered immune reaction, apoptosis and cellular death, including but not limited to the reproductive system, where reproductive issues associated with infertility become a worrisome nightmare [3]. Although harmful effects of crude oil are not gender-based, this present study focuses on the male reproductive system, as the male factor plays at least a partial role in the issue of fertility affecting families. It is estimated that approximately 7% of men globally have reported fertility challenges [4]. Conception is a function of healthy and functional sperm cells to a greater extent, where unhindered progressive movement of sperm is crucial. It has been evidentially documented that there are decreasing male reproductive potentials as characterized by low sperm count, abnormal morphology, asthenospermia and even azoospermia [5] [6]. The reproductive issues associated with the inability to conceive a child can be stressful and frustrating as many couples have not been able to conceive a child even though they have had frequent, unprotected sexual intercourse for not less than a year or longer.

Besides the presence of PAHs and other toxins in xenobiotic agents, which have been reported to have direct toxic effect on sperm, resulting in decreased numbers, as well as quality [7]-[9], conditions associated with metabolic disorder characterized by hyperglycaemia such as diabetes and obesity also impact on spermatogenesis and sperm functions. Diabetes mellitus (DM) remains one of the most complicated chronic disorders associated with dysfunctional glucose metabolism with global spread as a leading cause of morbidity and mortality [10] [11]. Complications of DM are associated with perturbation and interference with physiological

activities of many tissues, organs and systems of the body, where effect on male reproductive health is quite worrisome [12]. There have been clinical studies indicating the adverse role of hyperglycaemia in erectile and ejaculation dysfunction, reduced semen volume, and sperm parameters [13]-[15]. Aberrations in sperm parameters remain the leading cause of male factor infertility [16]. The pathogenesis of hyperglycemia-induced cellular damages in various organs has been linked to the formation of advanced glycation end product (AGE), activation of protein kinase C (PKC), and polyol pathway flux [17] [18]. The aforementioned mechanisms are all associated with oxidative stress, where the mitochondrial metabolic activities play a prominent role in producing elevated concentrations of reactive oxygen species. This in turn inhibits glyceraldehyde phosphate dehydrogenase (GAPDH) in the glycolytic pathway causing cellular and organ damages [18]. The pathway involving AGE formation is directly linked to disruptions in male fertility [19] [20], as its formation has been established to be accelerated under diabetic conditions [19] [20]. There is sufficient evidence in literature indicating the negative impact of AGE formation on testicular function, semen quality, and ultimately male fertility [19] [21]-[23]. Since the underlying mechanisms of hyperglycemia-induced male reproductive dysfunction are associated with induction of oxidative stress, the ability to balance out ROS concentrations in diabetic condition with anti-diabetic medication and antioxidant treatments might play some crucial roles in mitigating or ameliorating testicular, semen quality and indeed spermatozoa dysfunctions associated with DM to improve fertility capacity of sufferers, which informed the choice of caffeine in this study.

Distorted progressive movements coupled with abnormal morphologies of sperm are significant contributors to male infertility, these are manifested as defective sperm shape and structure, called teratospermia [4], such impairments hinder the sperm's ability to reach ovum and fertilize it. This study was therefore designed to investigate the mitigating effect of caffeine and Nigerian Bonnylight crude oil on sperm motility and morphology of diabetic rats.

## 2. Materials and Methods

The crude petroleum used in this study was obtained from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Nigeria following approval of a written application requesting for same. Alloxan monohydrate and caffeine were obtained from Sigma-Aldrich, USA. Distilled water was prepared by distillation at Derindam Research Institute of Biotechnology, Uyo, Akwa Ibom, Nigeria.

### 2.1. Acute Toxicity Test

The acute toxicity test for the NBLCO involved 25 mice weighing between 15 - 22 g divided into five groups with five mice per group. Mice in the five groups were administered intraperitoneally 10 ml/kg, 15 ml/kg, 20 ml/kg, 25 ml/kg and 30 ml/kg of body weight respectively, using Lorke's method [24].

$$LD_{50} = \sqrt{10 \times 20} = 14.14 \text{ ml/kg}$$

## 2.2. Experimental Animals

Male Wistar rats were reared in the Animal House of the Faculty of Basic Medical Sciences University of Uyo, Uyo, Nigeria and were kept in a well-ventilated section (experimental room) of the Animal House for a period of eighteen weeks. At the end of 18 weeks, ninety male Wistar rats weighing 180 - 200 g body weight were selected for the study. The ninety seemingly matured rats were selected and kept in an assigned experimental room for two weeks for acclimatization before commencement of experiment. After the two weeks of acclimatization, sixty rats were selected for the induction of diabetes.

## 2.3. Diabetes Induction

A total of sixty matured male Wistar rats were randomly selected for induction of diabetes. The animals were induced with alloxan-monohydrate at a single dose of 150 mg/kg body weight administered intraperitoneally. The state of diabetes was established 72 hours by measuring fasting blood glucose level from blood obtained from the tail of the animal using a glucometer (one-touch ultra, lifespan Inc), USA. Animals with fasting blood glucose of 200 mg/dL and above were considered diabetic, and forty of such rats were selected for the study.

## 2.4. Experimental Design and Treatment of Animals

A total of eighty adult male Wistar rats (180 - 200 g body weight) were randomly divided into eight groups, as presented in Table 1. In all cases, doses were applied daily for 28 days according to animal's most recent body weight. The experimental procedures involving the animals and their care were conducted in conformity with the approved guidelines by the Research and Ethical Committee of the Faculty of Basic Medical Sciences, University of Uyo, Uyo. Nigeria. The dose of NBLCO was taken as 20% of the lethal dose of 14.14 ml/kg.

**Table 1.** The grouping of the animals into various experimental groups and the specific treatments.

| Groups | Number of Animals | Treatments |
|---|---|---|
| I | 10 | Non-diabetic (3 mL/Kg body weight of distilled water) |
| II | 10 | Non-diabetic (20 mL/kg body weight of caffeine) |
| III | 10 | Non-diabetic (3 mL/Kg body weight of NBLCO) |
| IV | 10 | Non-diabetic (3 mL/Kg body weight of NBLCO plus 20 mL/kg body weight of caffeine) |
| V | 10 | Diabetic (3 mL/Kg body weight of distilled water) |
| VI | 10 | Diabetic (20 mL/Kg body weight of caffeine) |
| VII | 10 | Diabetic (3 mL/Kg body weight of NBLCO) |
| VIII | 10 | Diabetic (3 mL/Kg body weight of NBLCO plus 20 mL/kg body weight of caffeine) |

## 2.5. Collection of Blood Sample for Analysis

After the twenty-eight days of administration, the rats were anaesthetized with sodium pentobarbital at 50 mg/kg of body weight intraperitoneally. Sterile syringes of volume 5 mL and needles were used for the collection of blood samples by cardiac puncture. The total volume of blood collected on average was 4.7 mL, which was transferred into plain sample bottles and was allowed to stand for 2 hours to clot after which the serum was separated by centrifugation (RM-12 micro centrifuge, REMI, England) at 3000 rpm for 10 minutes at 25˚C. The serum obtained was stored at –20˚C until required for biochemical analysis (using Micro-Elisa methods).

The testes were harvested, from where the cauda epididymis was dissected out for the semen. Semen analysis was done using computer assisted semen analysis (CASA) in accordance with the Barratt (2007) [25] and WHO (1999) criteria [26].

## 2.6. Seminal Analysis Using Computer Aided System (CASA)

The cauda epididymis from each side of the testes was dissected out and several small cuts of about 1mm made and the tissue suspended in 1m of buffered formal saline to allow the spermatozoa to swim up. The assessment of sperm motility was done using computer-assisted semen analysis (CASA) in accordance with the method described by WHO [26] and Breanna Tilley [27]. The following measurements were obtained from the total cell detected and were used for the computation of other patterns of motility, these included total cell in sample ($10^6$/ml), Concentration ($10^6$/ml). Motile sperm, motile sperm rate %, concentration of motile sperm ($10^6$/ml), amplitude of lateral hunting (ALH) μm, wobbling (WOB) rate %, beating cilia frequency (BCF) in Hz, Linearity (LIN) in %, Straight forward line (SFR) %, progressivity (PR), non-progressive, immotility (IM), velocity of active path (VAP) μm/s, velocity of curve line (VCL) in μm/s, velocity of straight line (VSL) in μm/s, sperm of curve moving, sperm of Line moving, sperm of Line moving fast, concentration of curve moving (×$10^6$/ml), concentration of line moving ($10^6$/ml), concentration of line moving fast ($10^6$/ml), percentage of curve movement (%), percentage of curve movement (%), percentage of line moving (%), percentage of line fast (%), rate of line moving (%), rate of line moving fast (%) morphology count: normal cell, normality rate (%), anomality rate (%), defects, anomality of head, head anomality rate (%), anomality of body, body anomality rate (%), anomality of tail, tail anomality rate (%), mixed anomality, mixed anomality rate (%), red blood cell (RBC), white blood cell (WBC), epithelium, spermatocyte.

## 2.7. Statistical Analysis

Data were expressed as the mean ± standard error of the mean. Data were analyzed using one-way analysis of variance (ANOVA), results obtained were further subjected to post hoc using least significant deviation (LSD). Values of $p < 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Sperm Concentration Following Treatment with Caffeine and Nigerian Bonnylight Crude Oil for 28 Days

The results obtained following the administration of caffeine and Nigerian Bonnylight crude oil (NBLCO) is presented in Table 2.

Table 2. Comparing the spermatozoa concentration following administration caffeine and Nigerian Bonnylight crude oil.

| Groups | TSCD ($10^6$/mL) | TSCC ($10^6$/mL) | NSC (%) |
|---|---|---|---|
| ND Control | 445.25 ± 0.48 | 11.78 ± 0.17 | 91.25 ± 1.25 |
| ND Caf. | 207.80 ± 0.92$^{a}$ | 6.00 ± 0.13$^{a}$ | 22.40 ± 0.81$^{a}$ |
| ND CO | 149.00 ± 1.00$^{a,b}$ | 3.10 ± 1.00$^{a,b}$ | 15.50 ± 0.50$^{a,b}$ |
| ND Caf. + CO | 97.00 ± 0.38$^{a,b,c}$ | 2.47 ± 0.08$^{a,b,c}$ | 10.00 ± 0.22$^{a,b,c}$ |
| Diabetic | 117.08 ± 0.12$^{a,b,c,d}$ | 3.00 ± 0.11$^{a,b,d}$ | 11.20 ± 0.41$^{a,b,c}$ |
| D Caf. | 121.57 ± 1.45$^{a,b,c,d,e}$ | 4.29 ± 0.06$^{a,b,c,d,e}$ | 10.86 ± 0.34$^{a,b,c,e}$ |
| D CO | 87.63 ± 0.63$^{a,b,c,d,e,f}$ | 1.69 ± 0.10$^{a,b,c,d,e,f}$ | 9.75 ± 0.41$^{a,b,c,e}$ |
| D Caf. + CO | 88.50 ± 0.65$^{a,b,c,d,e,f}$ | 1.18 ± 0.12$^{a,b,c,d,e,f}$ | 4.00 ± 0.41$^{a,b,c,d,e,f,g}$ |

Note: a, b, c, d, e, f and g = versus groups ND Control, ND Caf., NDCO, ND Caf. + CO, Diabetic, D Caf., and D CO respectively at $p < 0.05$. TSCD = total sperm cell detected; TSCC = total sperm cell concentrated; NSC = normal sperm cells; ND = Non-diabetic; Caf. = Caffeine; CO = Nigerian Bonnylight crude oil; D = diabetic.

The results as presented in Table 2 showed that caffeine and NBLCO administration to both non-diabetic and diabetic rats significantly reduced the concentrations of total sperm cell detected (TSCD) and total sperm cell concentration (TSCC) as well as the percentage of normal sperm cell compared with ND control ($p < 0.05$). Similar significant ($p < 0.05$) reductions were reported in groups where caffeine was co-administered with NBLCO to non-diabetic and diabetic rats respectively.

Comparison of results between the diabetic animals treated with caffeine as well as group treated with NBLCO showed significant ($p < 0.05$) reductions in TSCD, TSCC AND NSC. But the reductions were more pronounced in the diabetic group treated with NBLCO as the mean values of this were significantly ($p < 0.05$) lower than diabetic rats treated with caffeine.

### 3.2. Results on the Morphology of Sperm Cell Following Administration Caffeine and Nigerian Bonnylight Crude Oil

The results of the impact of caffeine and Nigerian Bonnylight crude oil (NBLCO) administration on the morphology of sperm after 28 days of treatment are presented in Table 3.

The results as presented in Table 3 showed that caffeine and NBLCO administration to both non-diabetic altered sperm morphology significantly ($p < 0.05$) with distorted normal sperm cell morphology as head, body and tail anomaly rates

which were significantly higher compared to the non-diabetic untreated control group ($p < 0.05$).

**Table 3.** Comparing the morphology of sperm cell following administration caffeine and Nigerian Bonnylight crude oil.

| Groups | HAR (%) | BAR (%) | TAR (%) |
|---|---|---|---|
| ND Control | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 |
| ND Caf. | 6.00 ± 0.32[a] | 6.20 ± 0.49[a] | 4.60 ± 0.51[a] |
| ND CO | 12.50 ± 0.50[a,b] | 12.50 ± 0.50[a,b] | 12.00 ± 2.00[a,b] |
| ND Caf. + CO | 20.14 ± 0.14[a,b,c] | 22.86 ± 0.70[a,b,c] | 17.43 ± 0.69[a,b,c] |
| Diabetic | 22.24 ± 0.36[a,b,c,d] | 25.08 ± 0.92[a,b,c,d] | 19.65 ± 0.91[a,b,c,d] |
| D Caf. | 17.86 ± 0.46[a,b,c,d,e] | 23.71 ± 0.57[a,b,c,e] | 20.43 ± 0.43[a,b,c,d] |
| D CO | 25.00 ± 0.57[a,b,c,d,e,f] | 30.75 ± 0.49[a,b,c,d,e,f] | 19.38 ± 0.38[a,b,c,d] |
| D Caf. + CO | 28.75 ± 0.25[a,b,c,d,e,f,g] | 33.75 ± 0.63[a,b,c,d,e,f,g] | 41.25 ± 0.75[a,b,c,d,e,f,g] |

Note: a, b, c, d, e, f, and g = versus groups ND Control, ND Caf., NDCO, ND Caf. + CO, Diabetic, D Caf., and D CO respectively at $p < 0.05$.

The administration of caffeine to diabetic rats significantly ($p < 0.05$) reduced percentages of HAR, BAR and TAR compared with the diabetic group. The administration of NBLCO to diabetic rats on the other hand, significantly ($p < 0.05$) increase the percentages of HAR, BAR and but did not alter TAR significantly compared with the diabetic group.

### 3.3. Results on the Motility of Sperm Cell Following Administration Caffeine and Nigerian Bonnylight Crude Oil

The results for impact of caffeine and Nigerian Bonnylight crude oil (NBLCO) administration on sperm motility and all sperm parameters for progressive and efficient movement are presented in Table 4 and Table 5.

**Table 4.** Comparing the motility of sperm cells following administration caffeine and NBLCO.

| Groups | MSC (%) | PRS (%) | IMS (%) | NONPR (%) | LIN (%) | WR (%) |
|---|---|---|---|---|---|---|
| ND Control | 93.75 ± 0.63 | 81.75 ± 0.48 | 1.00 ± 0.00 | 1.75 ± 0.25 | 26.25 ± 0.63 | 1.00 ± 0.00 |
| ND Caf. | 58.80 ± 0.86[a] | 59.60 ± 0.68[a] | 13.38 ± 0.37[a] | 28.00 ± 0.71[a] | 10.00 ± 0.71[a] | 19.60 ± 0.24[a] |
| ND CO | 32.00 ± 1.00[a,b] | 19.00 ± 1.00[a,b] | 19.00 ± 1.00[a,b] | 37.00 ± 1.00[a,b] | 2.00 ± 0.00[a,b] | 26.50 ± 1.50[a,b] |
| ND Caf. + CO | 47.43 ± 0.61[a,b,c] | 16.29 ± 0.57[a,b,c] | 37.14 ± 0.46[a,b,c] | 34.29 ± 0.47[a,b,c] | 20.14 ± 0.14[a,b,c] | 30.29 ± 0.18[a,b,c] |
| Diabetic | 48.08 ± 0.16[a,b,c] | 49.06 ± 0.18[a,b,c,d] | 15.50 ± 0.59[a,b,c,d] | 30.02 ± 0.93[a,b,c,d] | 12.10 ± 0.51[a,b,c,d] | 21.80 ± 0.46[a,b,c,d] |
| D Caf. | 50.71 ± 0.29[a,b,c,d] | 54.71 ± 0.64[a,b,c,d,e] | 14.00 ± 0.69[a,b,c,d] | 21.29 ± 0.47[a,b,c,d,e] | 12.41 ± 0.34[a,c,d] | 20.29 ± 0.29[a,c,d] |
| D CO | 35.63 ± 0.50[a,b,c,d,e,f] | 12.38 ± 0.42[a,b,c,d,e,f] | 42.88 ± 0.72[a,b,c,d,e,f] | 35.63 ± 0.53[a,b,e,f] | 1.38 ± 0.18[a,b,d,e,f] | 36.88 ± 0.30[a,b,c,d,e,f] |
| D Caf. + D CO | 29.50 ± 0.50[a,b,c,d,e,f,g] | 7.00 ± 0.58[a,b,c,d,e,f,g] | 62.50 ± 0.50[a,b,c,d,e,f,g] | 61.00 ± 1.00[a,b,c,d,e,f,g] | 0.75 ± 0.25[a,b,f] | 55.25 ± 0.25[a,b,c,d,e,f,g] |

Note: a, b, c, d, e, f, and g = versus groups ND Control, ND Caf., NDCO, ND Caf. + CO, Diabetic, D Caf., and D CO respectively at $p < 0.05$. MSC = motile sperm cell; PR = progressive sperm; IMS = immotile sperm; NONPR = non-progressive sperm; LIN = linearity; WR = wobbling rate.

**Table 5.** Comparing the motility parameters of sperm cells following administration caffeine and NBLCO.

| Group | ND Control | ND Caf. | NDCO | ND Caf. + CO | Diabetic | D Caf. | DCO | D Caf. + CO |
|---|---|---|---|---|---|---|---|---|
| BCF (Hz) | 18.50 ± 0.65 | 8.40 ± 0.51$^{a}$ | 4.00 ± 0.00$^{a,b}$ | 2.43 ± 0.20$^{a,b}$ | 3.54 ± 0.30$^{a,b}$ | 3.43 ± 0.43$^{a,b,d}$ | 1.50 ± 0.19$^{a,b,c,e}$ | 0.75± 0.25$^{a,b,c,d,e,f}$ |
| SFL (%) | 10.50 ± 0.50 | 4.20 ± 0.49$^{a}$ | 1.00 ± 0.00$^{a,b}$ | 1.43 ± 0.20$^{a,b}$ | 2.54 ± 0.31$^{a,b,c}$ | 2.29± 0.18$^{a,b,c,d}$ | 1.25 ± 0.16$^{a,b,e}$ | 0.75± 0.25$^{a,b,d,e,f}$ |
| VAP (μm/s) | 21.25 ± 0.63 | 5.20 ± 0.20$^{a}$ | 2.00 ± 0.00$^{a,b}$ | 1.29 ± 0.18$^{a,b}$ | 2.40 ± 0.29$^{a,b,d}$ | 1.43 ± 0.20$^{a,b}$ | 0.38 ± 0.18$^{a,b,c,d,e}$ | 0.79 ± 0.25$^{a,b,e,g}$ |
| VCL (μm/s) | 18.75 ± 0.63 | 5.00 ± 0.45$^{a}$ | 2.00 ± 0.00$^{a,b}$ | 1.43 ± 0.02$^{a,b}$ | 2.54 ± 0.13$^{a,b,d}$ | 1.43 ± 0.03$^{a,b,e}$ | 0.75 ± 0.16$^{a,b,c,e}$ | 0.75 ±0.25$^{a,b,d}$ |
| VSL (μm/s) | 11.25 ± 0.25 | 3.40 ± 0.24$^{a}$ | 1.00 ± 0.00$^{a,b}$ | 1.29 ± 0.18$^{a,b}$ | 2.40 ± 0.29$^{a,b,c}$ | 1.43 ± 0.02$^{a,b}$ | 0.75 ± 0.20$^{a,b,d,e}$ | 0.75 ± 0.25$^{a,b,e}$ |
| CCMS ($10^{6}$/mL) | 11.25 ± 0.63 | 7.40 ± 0.24$^{a}$ | 1.50 ± 0.50$^{a,b}$ | 1.14 ± 0.14$^{a,b}$ | 2.25 ± 0.25$^{a,b,d}$ | 1.43 ± 0.20$^{a,b}$ | 1.13 ± 0.13$^{a,b,e}$ | 0.75 ± 0.25$^{a,b,e}$ |
| CLMS ($10^{6}$/mL) | 11.00 ± 0.71 | 4.20 ± 0.58$^{a}$ | 1.00 ± 0.00$^{a,b}$ | 1.14 ± 0.14$^{a,b}$ | 1.14 ± 0.14$^{a,b}$ | 1.43 ± 0.20$^{a,b}$ | 0.88 ± 1.25$^{a,b}$ | 0.76 ± 0.25$^{a,b}$ |
| CFLMS ($10^{6}$/ml) | 11.25 ± 1.25 | 2.20 ± 0.20$^{a}$ | 2.00 ± 0.00$^{a}$ | 1.29 ± 0.18$^{a}$ | 1.29 ± 0.20$^{a,b}$ | 1.57 ± 0.20$^{a}$ | 1.23 ± 0.13$^{a}$ | 0.78 ± 0.25$^{a,b}$ |

Note: a, b, c, d, e, f, and g = versus groups ND Control, ND Caf., NDCO, ND Caf. + CO, Diabetic, D Caf., and D CO respectively at $p < 0.05$. BCF = beating cilia frequency; SFL = straight forward line; VAP = velocity of active path; VCL = velocity of curve line; VSL = velocity of straight line; CCMS = concentration of curve moving spermatozoa; CLMS = concentration of line moving spermatozoa; CFLMS = concentration of fast line moving spermatozoa.

The results showed that independent administration of caffeine and NBLCO significantly ($p < 0.05$) reduced the ability of the sperm cell to move efficiently by significantly ($p < 0.05$) reducing the percentages of motile sperm (MS), progressivity and linearity, while the percentages of immotile and non-progressive sperm cell as well as wobbling rates were significantly ($p < 0.05$) increased with respect to the control group. The induction of diabetes presented a similar pattern of results. The administration of caffeine to diabetic rats did not alter sperm motility significantly, but the administration of NBLCO significantly ($p < 0.05$) distorted sperm motility by significantly ($p < 0.05$) reducing percentages of MSC, PRS and linearity while significantly ($p < 0.05$) increasing percentages of immotile sperm, non-progressive sperm and wobbling sperm.

The results showed that independent administration of caffeine and NBLCO significantly ($p < 0.05$) reduced the ability of the sperm cell to move efficiently by significantly ($p < 0.05$) reducing all parameters that favour progressive sperm advancement, as presented in Table 5.

## 4. Discussion

The mitigating effect of caffeine on Nigerian Bonnylight crude oil (NBLCO) impaired sperm motility and oxidative stress of diabetic Wistar rats was investigated in this study. The results obtained following the administration of caffeine and NBLCO to male Wistar rats obtained in this present study showed that independent administration of caffeine and NBLCO to non-diabetic rats significantly altered sperm parameters, such as concentration, morphology and motility. These alterations were aggravated in diabetic rats, which collaborates with the report of He *et al.* [28], where diabetes mellitus was reported to instigate changes in semen quality. And when NBLCO was administrated to the diabetic rats, this condition was made worst; giving credence to the fact that exposure to crude oil and indeed

polycyclic aromatic hydrocarbons (PAHs) can directly and indirectly impact reproductive health by a decline in fertility of human and wildlife [29]-[32]. Reduction in total sperm concentrations as indicated by the total sperm cell detected (TSCD) and total sperm cell concentration (TSCC), as well as the percentage of normal sperm cells, was observed in this study. A similar pattern of result was observed when caffeine was co-administered with NBLCO to non-diabetic and diabetic rats respectively, with a more pronounced reduction in the diabetic group treated with NBLCO, as the mean value was significantly lower than those rats treated with caffeine.

The administration of caffeine to non-diabetic rats altered sperm morphology significantly with distorted morphology of the head, body and tail. The independent administration of NBLCO to diabetic rats aggravated these distortions even more, whereas independent administration of caffeine significantly reversed these effects by reducing percentages of distorted head, body and tail morphology.

The independent administration of caffeine and NBLCO reduced sperm cell ability to make progressive movement by significantly reducing all indices that promote sperm motility as indicated by the reduced percentages of motile sperm (MS), progressivity and linearity, while the percentages of immotile and non-progressive sperm cell as well as wobbling rates were significantly increased. Similar pattern of results was recorded in diabetic rats, which was aggravated by NBLCO administration. It is important to report here that while administration of caffeine to diabetic rats did not alter sperm motility significantly, the administration of NBLCO significantly distorted sperm motility by significantly reducing percentages of motile sperm cell, progressive sperm and linearity. The aforementioned indices promote sperm motility. It furthermore significantly increases percentages of immotile sperm, non-progressive sperm and wobbling sperm to promote infertility. The outcome of this present study agrees with similar reports in literature, where NBLCO recorded reduced sperm count and motility as well as distorted morphology in rat model [33]-[36]. The viability and quality of such sperm can never be trusted, as these characteristics depend on the aforementioned parameters. And so does male fertility, which in this context is the ability of the sperm to efficiently advance towards an ovum for proper interaction and penetration of the sperm nucleus to fuse with that of the ovum for fertilization. It appears that the entire parameters favouring motility may have been distorted by NBLCO toxicity and diabetes. It has been postulated that sperm motility is a critical parameter determining the quality of semen, which has been used as reliable predictive factor for male fertility success [37] [38]. And this makes motility a critical factor for successful fertilization as only sperm that can progressively embark on forward-movement can successfully navigate the female mucus-rich genital tract to fertilize an ovum, and so, unsuccessful fertilization can occur due to impaired sperm motility. The findings of this study indicated that both NBLCO administration and diabetes mellitus increase the percentage of immotile sperm as well as promoting those parameters that do not promote motility, this can in turn promote infertility by induction

of asthenospermia, which is characterized by reduced or no motility of sperm cells in a fresh semen ejaculate as reported in the present study. Immotile sperm lacks the capacity to navigate through the cervical mucus to reach the ovum. The navigation of the mucus-rich female genital tract requires a viable sperm with viable flagellum for efficient swimming, as altered structure of the flagellum can impair motility, which appears to be the effect of NBLCO administration in this study. The most probable mechanism of cellular injuries of NBLCO could be its ability to induce oxidative stress, inflammation and apoptosis, as reported in literature [33]-[36].

The progressive advancement of sperm is a critical factor in determining their viability and the capacity to fertilize an ovum. The findings of this study have shown that diabetes mellitus and administration of environmental stressors such as NBLCO have indicated significant interference with sperm function and viability, which negatively impacted sperm motility. This has been documented in this study by a significant reduction in all parameters that promote progressive advancement of sperm while significantly increasing those parameters that do not promote motility, which the intervention with caffeine significantly reversed. It can be concluded that DM and NBLCO administration have shown the tendency to promote male infertility by reducing sperm motility and adversely altering the morphology of sperm in male Wistar rats, which was ameliorated by caffeine intervention.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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