Ameliorative Effects of N-Acetyl Cysteine and Zinc Sulfate on Reproductive Dysfunction Induced by Short-Term Crude Oil Exposure in Male Wistar Rats

ABSTRACT

Background: Crude oil contamination by accidental or voluntary ingestion is prevalent in the Niger Delta region of Nigeria. One of the possible impacts of crude oil contamination is reproductive toxicity leading to infertility. The present study evaluated the potential protective effects of N-Acetyl Cysteine (NAC) and Zinc Sulfate ($ZnSO_4$)in mitigating reproductive dysfunction caused by short-term Bonny Light Crude oil (BLCO) exposure in male Wistar rats.

Materials and Methods: Fifty (50) male Wistar (180 – 200g) were used for the study. They were divided into ten (10) groups of five (5) animals each. Groups I and II served as the control and negative control and received distilled water and BLCO (600mg/kg) respectively while groups III to X served as the experimental groups and received NAC (100 and 200mg/kg) and $ZnSO_4$ (0.5mg/kg and 1mg/kg) orally for three (3) weeks. Animals were euthanized, and blood and semen were collected for biochemical and seminal analysis.

Results: The results of this current study show that BLCO exposure caused a disruption in reproductive functions in rats by decreasing reproductive hormones and semen quality parameters in Wistar rats. NAC and $ZnSO_4$ administration significantly improved semen quality parameters by improving sperm count, sperm motility and morphology in experimental rats.

Conclusion: Evidence from the present study suggests that NAC and ZnSO4 protected the testes, preventing reproductive alterations linked to BLCO exposure

Keywords; Crude Oil, Bonny crude light oil, N-Acetyl Cysteine, Zinc Sulphate, reproductive dysfunction

INTRODUCTION

Petroleum pollution is a prevalent issue that can occur both accidentally and during routine operations wherever oil is produced, transported, stored, processed, or utilized, whether on land or at sea. On land, petroleum products often represent a significant portion of the contaminants found at polluted sites [1, 2]. Despite the economic importance of crude oil in Nigeria, its exploration has brought about many pollution problems, caused by oil spillage which is a common occurrence in Nigeria [3, 4]. Interestingly, the traditional practice of consuming crude oil for its purported medicinal benefits is becoming more widespread [5]. They are directly used as curative agents for anti-poisoning (snake venom antidotes), anti-convulsion, treatment of skin infection or indirectly by eating marine animals found in surrounding coastal waters as a source of protein [6]. Bonny light crude oil (BLCO) is used in combination with olive oil in folklore medicine in some parts of the Niger Delta region of Nigeria to treat burns, gastrointestinal

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disorders, ulcers, witchcraft attacks and poisoning [6-8].Several chemicals contained in BLCO have been identified as endocrine disruptors, resulting in unwanted reproductive malfunction and developmental disorders [7, 9-12].

Infertility is one of the major health-therapeutic problems in different societies [13], affecting between fifty and eighty million couples at some point in their reproductive lives globally [14]. Approximately 8% of men seek medical help for fertility-associated problems [15]. An estimated 3-4 million Nigerian couples have fertility-associated problems [16]. Infertility affects about 20-30% of couples in Nigeria and around the world, irrespective of their race or ethnicity [17]. Studies have shown that BLCO significantly impairs reproductive functions by causing reduced sperm count, sperm motility and normal morphology within seven days of administration [1, 10, 11]. The mechanism behind the reproductive damage caused by BLCO is associated with the generation of free radicals, reactive oxygen and reactive nitrogen species which overwhelm cellular antioxidant systems and induce cellular damage [18]. Oxidative stress occurs when the balance between the production of reactive species and the antioxidant defence system is disrupted [19-21]. Cellular injury is primarily due to the inability of the antioxidants to neutralize the effects of the oxygen radicals, which have ultimately led to infertility[18]. Antioxidants are molecules that prevent oxidative damage by scavenging reactive oxygen species (ROS) or inhibiting their production, thereby limiting or preventing the oxidation of other molecules [22-25]. Antioxidant compounds such as N-acetylcysteine (NAC) plays an important role in the protection of cell constituents from oxidative stress [26]by acting as a cysteine supplier, maintains and increases the intracellular levels of glutathione [27, 28]. Zinc (Zn) is another important trace element found in small amounts in a variety of cells and tissues of organisms, it is a cofactor of more than 300 enzymes [29]. It is reported to be necessary for signal transduction, DNA replication, RNA polymerases, protein synthesis, growth processes and various metabolic processes [30]. Various studies have shown that zinc sulfate may act as an antioxidant [31], capacitating agent [32, 33], membrane-stabilizing factor [34] and sperm motility factor [35, 36]. Research is scarce on the potential ameliorative effects of these key antioxidant compounds on reproductive dysfunction caused by crude oil exposure. This study aims to address this gap by evaluating the potential protective role of N-Acetyl Cysteine and Zinc Sulfate in mitigating reproductive dysfunction induced by short-term crude oil exposure in male Wistar rats.

MATERIALS AND METHODS

Source of Crude, Drugs and Research Animals

Bonny light crude oil was obtained from the Nigerian National Petroleum Corporation (NNPC) Warri, Nigeria. N-acetyl cysteine was purchased from Sigma Aldrich, USA (A7250-10G) while Zinc sulfate was sourced from a local pharmaceutical shop in Port Harcourt, River State. Fifty (50) male Wistar (180 – 200g) were sourced from the Animal house of the Department of

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Physiology, Delta State University, Abraka and used for the study. They were allowed three (3) weeks of acclimatization under standard animal husbandry conditions. The animals were housed in clean, well-ventilated wooden cages under optimal conditions, including a 12-hour light/dark cycle, a temperature range of 28–31ºC, and humidity levels of 45–50%. They had unrestricted access to standard rat pellets and tap water. The animals were divided into ten (10) groups of five (5) animals each and received BLCO, NaC and $ZnSO_4$ [37, 38] orally via orogastric cannula between 8am and 10am daily for three (3) weeks according to the following protocol:

Group I	Distilled water only (control)
Group II	BLCO (600mg/kg) only (negative control)
Group III	NAC (100mg/kg) only
Group IV	NAC (200mg/kg) only
Group V	NAC (100mg/kg) + BLCO (600mg/kg)
Group VI	NAC (200mg/kg) + BLCO (600mg/kg)
Group VII	ZnSO4 (0.5 mg/kg) only
Group VIII	ZnSO4 (1mg/kg) only
Group IX	ZnSO4 (0.5mg/kg) + BLCO (600mg/kg)
Group X	ZnSO4 (1mg/kg) + BLCO (600mg/kg)

Sample Collection and Biochemical aboratory Assay
At the end of the experimental period, the animals were fasted overnight after which they were euthanized by cervical dislocation and cardiac puncture was used to collect blood sample for hormonal and biochemical analysis. The enzyme linked immunosorbent assay technique was used for follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estrogen and prolactin estimation using standard ELIZA kits (Cal biotech Inc. California). The procedure for the estimation of serum concentration of each hormone was carried out according to the kits' manual as earlier described by Khourdaji et al[39].

Semen Analysis
For sperm count, the caudal epididymis was homogenized in formal-saline and the sperm count was carried out using the improved Neubauer counting chamber (LABART, Germany). The sperm count was examined under the light microscope at a magnification of x40 while evaluating different fields and was calculated using the formula described by Omirinde et al.[40]. Sperm motility was determined by conventional method as described by Khatun et al., [41]. After the sperm had been squeezed on the pre-warmed slide, two drops of warm 2.9% sodium citrate were added to it, then covered with a cover slip and examined under the microscope using an X40 objective with reduced light. Sperm Morphology was done by collecting a thin smear of the sperm sample on a clean slide, fixed with 95% ethanol and allowed to air dry. The fixed slide was then sequentially immersed into different concentrations of ethanol and appropriately stains namely Harris haematoxylin, G-6 orange stain and EA-50 green stain for one minute. The slide was then examined microscopically at a magnification of ×40, 200 sperm were assessed and the sperm abnormalities were expressed in percentages [40, 42]. Sperm Viability (Live/Death Raito)

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was done by observing the percentage of spermatozoa in a unidirectional progressive movement over a field on a slide under the light microscope fitted with a camera using the Eosin/Nigrosin stain[40].

Statistical Analysis

Data obtained from this study was analyzed using Graph pad Prism 8 Biostatistics software (Graph pad Software, Inc., Lajolla, USA version 8.0). All data was presented as Mean±SEM. Further analysis was done by one analysis of variance (ANOVA) and followed by a post hoc test (Bonferroni) for multiple comparisons. The level of significance for all tests was set at $p < 0.05$.

Ethical Considerations

The animals were treated in accordance with the highest ethical standards for animal experimentation. The research protocol and study design were approved by the University of Port Harcourt Research Ethics Committee.

RESULTS

Table 1: Role of NAC and $ZnSO_4$ administration on Testosterone, Estrogen, Follicle stimulating hormone, Luteinizing hormone and Prolactin level of BLCO-exposed Wistar rats

Groups/Doses(/kg bw)	Testosterone (ng/ml)	Estrogen (pg/ml)	FSH (miU/ml)	LH (miU/ml)	Prolactin (µg/L)
Control	78.03±1.28	0.61±0.01	1.68±0.01	3.92±0.04	6.23±0.13
BLCO $_{(600mg/kg)}$	$42.43±1.37^{*}$	$0.39±0.02^{*}$	$0.23±0.02^{*}$	$1.34±0.03^{*}$	$4.52±0.27^{*}$
NAC $_{(100mg/kg)}$	$75.27±0.87^{*\#}$	$0.57±0.01^{\#}$	$1.09±0.03^{*\#}$	$2.69±0.08^{*\#}$	$6.05±0.10^{\#}$
NAC $_{(200mg/kg)}$	$72.04±2.17^{\#}$	$0.41±0.01^{*\phi}$	$1.24±0.01^{*\#}$	$2.76±0.07^{*\#}$	$5.09±0.20^{*\phi}$
$NAC_{(100mg/kg)}$+ $BLCO_{(600mg/kg)}$	$69.04±0.62^{*\#\beta}$	$0.47±0.01^{*\alpha}$	$0.19±0.01^{*\alpha\beta}$	$2.14±0.01^{*\#\beta}$	$5.88±0.13^{\#}$
$NAC_{(200mg/kg)}$ + $BLCO_{(600mg/kg)}$	$74.24±0.47^{\#}$	$0.57±0.02^{\#\beta}$	$0.22±0.01^{*\alpha\beta}$	$2.32±0.07^{*\#\alpha}$	$5.68±0.34^{\#}$
$ZnSO_{4(0.5mg/kg)}$	$60.48±0.79^{*\#}$	$0.46±0.01^{*}$	$1.02±0.02^{*\#}$	$2.65±0.05^{\#}$	5.53±0.15
$ZnSO_{4(1mg/kg)}$	$73.43±0.41^{\phi\#}$	$0.54±0.02^{\#}$	$0.87±0.01^{*\#\phi}$	$3.43±0.05^{*\#}$	$5.83±0.26^{\#}$
$ZnSO_{4(0.5mg/kg)}$ + $BLCO_{(600mg/kg)}$	$36.22±1.49^{*\#ab}$	$0.52±0.01^{*\#}$	$0.23±0.01^{*ab}$	$2.81±0.14^{*\#ab}$	5.31±0.26
$ZnSO_{4(1mg/kg)}$ + $BLCO_{(600mg/kg)}$	$53.60±0.80^{*\#abc}$	$0.54±0.01^{\#}$	$0.13±0.02^{*\#ab}$	$2.84±0.24^{*\#ab}$	5.52±0.12

Values are expressed as Mean±SEM (n = 5) (ANOVA followed by Turkeys test).
BLCO. (BLCO=Bony Light Crude Oil. NAC = N-acetylcysteine. $ZnSO_4$ = Zinc Sulfate).
**P<0.05*: significantly different when compared with the control group.
$^{\#}$*P<0.05*: Significantly different when compared with BLCO.
$^{\alpha}$*P<0.05*: significantly different when compared with $NAC_{\mathbf{a}}$ (100mg/kg).
$^{\beta}$*P<0.05*: significantly different when compared with $NAC_{\mathbf{b}}$ (200mg/kg).
$^{\phi}$*P<0.05*: Dose-dependent significant difference.
^{a}*P<0.05*: significantly different when compared with $ZnSO_{4\mathbf{a}}$(0.5mg/kg).
^{b}*P<0.05*: significantly different when compared with $ZnSO_{4\mathbf{a}}$(1mg/kg).
^{c}*P<0.05*: significantly different when compared with ZnSO4a(0.5mg/kg)

Table 1 shows the role of NAC and $ZnSO_4$ administration on testosterone, estrogen, follicle-stimulating hormone, Luteinizing hormone and Prolactin level of BLCO-exposed Wistar rats. Our data show that BLCO exposure caused reduced testosterone levels when compared with the control ($p<0.05$). The group co-treated with NAC (both doses) had a significant increase in testosterone levels when compared with the short-term BLCO-exposed group only. Also, a dose-dependent increase in testosterone levels was observed among $ZnSO_4$ administered group when compared to the BLCO-exposed group. Testosterone level was significantly decreased in NAC_a +BLCO group when compared with NAC_b alone administered group ($p<0.05$). A Dose-dependent increase in testosterone level was observed in $ZnSO_4$ administered group when compared with the exposed group ($p<0.05$). Furthermore, the testosterone level was also significantly decreased in $ZnSO_4$ + BLCO administered group when compared with $ZnSO_{4a}$ and $ZnSO_{4a}$ respectively ($P<0.05$). Similarly, BLCO exposure demonstrated significantly decreased estrogen levels in experimental animals when compared with the control group ($p<0.05$). A significant increase in estrogen level was observed in the group co-treated with NAC_b+ BLCO when compared with the short-term BLCO-exposed group ($p<0.05$). Also, estrogen level was significantly increased in the experimental group co-treated with $ZnSO_4$ (both doses) and BLCO when compared with the short-term BLCO-exposed group alone ($p<0.05$). Although the NAC_a+ BLCO group demonstrated a significant decrease in estrogen level, estrogen level was significantly increased in the experimental group co-treated with NAC_b+ BLCO when compared with NAC_a alone and NAC_b respectively. BLCO exposure significantly decreased FSH levels when compared with the control group ($p<0.05$). Co-treatment BCLO-exposed rats with NAC and $ZnSO_4$ did not cause any significant improvement in the FSH level when compared with BLCO-exposed rats alone. However, a significant decrease in FSH level was observed for rats exposed to BLCO and co-treated with NAC and $ZnSO_4$ when compared with the NAC alone and $ZnSO_4$ alone administered group respectively ($p<0.05$). Luteinizing hormone level was significantly decreased in BLCO-exposed rats when compared with the control group ($p<0.05$). However, co-treatment of BLCO-exposed rats with NAC and $ZnSO_4$ (both doses) significantly increased LH levels when compared with short-term BLCO-exposed rats. Furthermore, a significant decrease in LH level is also observed for short-term BLCO-exposed rats co-treated with NAC and $ZnSO_4$ when compared with NAC alone administered group and $ZnSO_4$ alone administered group respectively. BLCO exposure also causeda significant decrease in the prolactin level of Wistar rats when compared with the control group ($p<0.05$). A significant increase in the prolactin level is observed in rats co-treated with NAC and BLCO (both doses) when compared with rats exposed to short-term BLCO only ($p<0.05$).

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Table 2: Role of NAC and $ZnSO_4$ administration on sperm count, motility, morphology, and viability of BLCO-exposed Wistar rats

Groups/Doses(/kg bw)	**Sperm Count ($x10^{-6}$ cells/mm^3)**	**Sperm Motility (%)**	**Sperm Morphology (%)**	**Sperm Viability (%)**
Control	110.20 ± 1.77	76.24 ± 1.22	72.80 ± 0.58	81.60 ± 1.03
BLCO $_{(600mg/kg)}$	$76.48\pm3.86^{*}$	$42.38\pm0.95^{*}$	$43.40\pm0.97^{*}$	$46.20\pm1.35^{*}$
NAC $_{(100mg/kg)}$	$94.40\pm1.69^{*\#}$	$58.82\pm0.68^{*\#}$	$56.60\pm0.81^{*\#}$	$72.20\pm0.66^{*\#}$
NAC $_{(200mg/kg)}$	$109.80\pm2.63^{\#\phi}$	$63.40\pm2.15^{*\#}$	$68.20\pm1.15^{\#\phi}$	$69.40\pm1.93^{*\#}$

$NAC_{(100mg/kg)}$ + $BLCO_{(600mg/kg)}$	$97.40 \pm 2.61^{*\#}$	$46.40 \pm 0.24^{*\beta}$	$57.20 \pm 0.73^{*\#\beta}$	$57.80 \pm 1.28^{*\#\alpha\beta}$
$NAC_{(200mg/kg)}$ + $BLCO_{(600mg/kg)}$	$106.70 \pm 2.79^{\#}$	$51.80 \pm 0.58^{*\#\beta}$	$67.20 \pm 1.35^{*\#\alpha}$	$61.00 \pm 1.51^{*\#\alpha\beta}$
$ZnSO_{4(0.5mg/kg)}$	$79.70 \pm 2.80^{*}$	$47.37 \pm 1.07^{*}$	$60.40 \pm 0.81^{*\#\phi}$	$57.80 \pm 1.28^{*\#}$
$ZnSO_{4(1mg/kg)}$	$89.13 \pm 2.58^{*}$	$60.32 \pm 0.03^{*\#\phi}$	$66.60 \pm 0.92^{*\#}$	$73.60 \pm 1.96^{*\#}$
$ZnSO_{4(0.5mg/kg)}$ + $BLCO_{(600mg/kg)}$	$105.30 \pm 2.24^{\#a}$	$59.66 \pm 2.52^{*\#a}$	$62.00 \pm 1.37^{*\#}$	$55.60 \pm 1.12^{*\#b}$
$ZnSO_{4(1mg/kg)}$ + $BLCO_{(600mg/kg)}$	$84.11 \pm 1.80^{*bc}$	$67.55 \pm 1.23^{*\#abc}$	$66.60 \pm 1.12^{*\phi\#a}$	$73.60 \pm 0.74^{*\phi\#a}$

Values are expressed as Mean±SEM (n = 5) (ANOVA followed by Turkeys test).
BLCO. (BLCO=Bony Light Crude Oil. NAC = N-acetylcysteine. $ZnSO_4$ = Zinc Sulfate).
**P<0.05*: significantly different when compared with the control group.
$^{\#}$*P<0.05*: Significantly different when compared with BLCO.
$^{\alpha}$*P<0.05*: significantly different when compared with $NAC_{\mathbf{a}}$ (100mg/kg).
$^{\beta}$*P<0.05*: significantly different when compared with $NAC_{\mathbf{b}}$ (200mg/kg).
$^{\phi}$*P<0.05*: Dose dependent significant difference.
^{a}*P<0.05*: significantly different when compared with $ZnSO_{4\mathbf{a}}$(0.5mg/kg).
^{b}*P<0.05*: significantly different when compared with $ZnSO_{4\mathbf{a}}$(1mg/kg).
^{c}*P<0.05*: significantly different when compared with ZnSO4a(0.5mg/kg)

Table 2 shows the effect of NAC and $ZnSO_4$ administration on sperm count, motility, morphology, and viability of BLCO-exposed Wistar rats. Our findings show that Wistar rats exposed to BLCO for three (3) weeks demonstrated significantly decreased sperm count when compared with the control group (p<0.05). Co-treatment of BLCO-exposed Wistar rats with NAC (both doses) and $ZnSO_{4a}$ significantly increased the sperm count when compared with Wistar rats administered with only BLCO. There was a dose-dependent increase in the sperm count of Wistar rats treated with NAC. Also, the study finds that Wistar rats exposed to BLCO demonstrated significantly decreased sperm motility when compared with the control group (p<0.05). However, co-treatment of BLCO-exposed Wistar rats with NAC_b and $ZnSO_4$ significantly increased the sperm motility of Wistar rats when compared with Wistar rats exposed to only BLCO respectively (p<0.05). Although Wistar rats co-treated with NAC + BLCO demonstrated significantly decreased sperm motility when compared with Wistar rats administered with only NAC (P<0.05), however, Wistar rats co-treated with $ZnSO_4$ + BLCO demonstrated increased sperm motility when compared with Wistar rats administered with $ZnSO_4$ only (p<0.05). Our results also that sperm morphology was affected by BLCO exposure as BLCO-exposed Wistar rats demonstrated significantly decreased spermatozoa with normal morphology when compared with the control group (p<0.05). Also, when co-treated with NAC + BLCO and $ZnSO_4$ + BLCO, there was a dose-dependent significant increase in spermatozoa with normal morphology when compared with Wistar rats exposed to only BLCO (p<0.05). A significant decrease in spermatozoa with normal morphology is also observed with Wistar rats co-treated with NAC + BLCO and $ZnSO_4$ + BLCO when compared with the experimental group administered with only NAC and $ZnSO_4$ respectively. Wistar rats exposed to BLCO demonstrated a significant reduction in sperm viability when compared with the control group. However, when co-treated with NAC + BLCO and $ZnSO_4$ + BLCO, there was a significant increase in the viable sperm cells when compared with Wistar rats exposed to only BLCO (p<0.05). The percentage of viable sperm cells is also significantly reduced among NAC + BLCO and $ZnSO_4$ + BLCO when compared with NAC alone and $ZnSO_4$ alone groups

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respectively ($P<0.05$). Wistar rats exposed to BLCO for 7 weeks demonstrated a significant decrease in sperm viability when compared with the control group. However, co-treated groups NAC + BLCO and $ZnSO_4$ + BLCO recorded a significant increase in the viable sperm cells when compared with Wistar rats exposed to only BLCO. Furthermore, the percentage of viable sperm cells is also significantly reduced among NAC + BLCO and $ZnSO_4$ + BLCO groups when compared with NAC alone and $ZnSO_4$ alone groups respectively. There is also a dose-dependent increase in viable sperm in Wistar rats treated with $ZnSO_4$.

DISCUSSION

Crude oil pollution can occur accidentally during transportation or as a routine consequence of production and processing. While accidental ingestion may result from contamination, there are instances where crude oil is voluntarily consumed by humans due to its purported medicinal benefits. Both accidental and voluntary ingestion are common in Nigeria's Niger Delta region. This study assessed the potential protective effects of N-Acetyl Cysteine and Zinc Sulfate in mitigating reproductive dysfunction caused by short-term crude oil exposure in male Wistar rats.

Effect of NAC and $ZnSO_4$ on reproductive hormones of BLCO-exposed Wistar rats

The result from the present study shows that BLCO significantly reduced reproductive hormones in Wistar rats after three weeks when compared with the control group ($p<0.05$, Table 1). This shows that BLCO contamination and consumption could negatively regulate releasing of gonadotropins through the pituitary gland. FSH and LH are the main factors needed for the development of testicles and for proper stimulation of releasing of reproduction hormones like testosterone, estrogen and progesterone. The decrease in the level of these bio-substances in rats treated with BLCO could mean a negative effect on the anterior pituitary gland which influences normal control and regulation of processes involved in secreting these reproduction-related hormones. Findings from the present study are consistent with studies done by [7, 11, 12, 43, 44]. The reduced level of testosterone in BLCO-administered rats could be an indicator of the chemical toxic level in the male reproductive organ, which is crucial and required to sustain spermatogenesis and also to maintain their function and structure as regards male sex glands [45]. Our study observed that co-administration of BLCO with NAC and $ZnSO_4$ substantially increased all reproductive hormone levels. This finding is consistent with the study by Nashwa *et al.,* [46]where a notable increase in testosterone, LH and FSH levels was observed due to NACs. NACs are precursors of L-cysteine which leads to glutathione elevation synthesis. They are antioxidant agents and common nutrition supplements, applied anti-oxidant*in-vivo*and*in-vitro*. It acts directly as a scavenger of free radicals, especially oxygen. It is equally recommended as a possible remedial alternative to several illnesses that arise from the formation of oxygenated radicals [47]. NACs could prevent the negative impact of different toxins like arsenic, lead and cadmium on male reproduction [48, 49]. A similar result also shows that co-administration of $ZnSO_4$ with BLCO significantly increased all reproductive hormones (Table 1). This corroborates findings from previous studies of Egwurugwu *et al.,* [36] on the effect of $ZnSO_4$ on male reproductive hormones. Zinc supplementation activates the secretion and action of testosterone and can lead to increased efficiency of spermatogenic machinery and increased number of germ cells in the seminiferous tubules [50]. The reduction in serum levels of FSH and non-significant effect on Luteinizing hormone (LH) following the increased levels of testosterone may be attributed to the negative feedback effect of testosterone on the

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hypothalamus which in turn causes a decrease in the secretion of FSH and LH by the anterior pituitary gland. This observed improvement in the values of testosterone in the $ZnSO_4$-supplemented group might be due to the stimulating effect of ZnSO4 on testicular steroidogenesis as $ZnSO_4$has been shown to affect testicular functions by activating the adenyl cyclase system, which stimulates testosterone synthesis [51].

Effect of NAC and $ZnSO_4$ on sperm parameters of BLCO-exposed Wistar rats

Results from the present study show that BLCO in a dose-dependent manner significantly decreased sperm quality parameters (sperm count, motility, morphology and viability) when compared with control ($p<0.05$, Table 2). The findings from the present study are consistent with the result ofFarombi*et al*[11] where it was demonstrated that BLCO caused a reduction in epididymal sperm number (ESN), daily spermatozoa production (DSP),and sperm motility. Also, previous studies have observed a reduction insperm count, motility and abnormal morphology in BLCO-exposed rats[10, 52, 53].The study also demonstrated that NAC co-administration significantly improved sperm count, motility, viability and sperm morphology) when compared with the administered group ($p<0.05$, Table 2).Several other studies have highlighted the ability of NAC to improve sperm count, motility and morphology after paracetamol, arsenic and aluminium sulfate-induced oxidative stress and reproductive toxicity in male albino rats [48, 54-57]. Similarly, the co-administration of BLCO and $ZnSO_4$, showedsignificantly improved sperm count, motility, viability and sperm morphology when compared with the BLCO-administered group ($p<0.05$). Zinc supplementation has been demonstrated to promote the transformation of sperm nuclear protein (i.e., from lysine to arginine) and inhibit the premature depolymerization of the sperm nucleus, improve sperm motility and semen quality in infertile patients without obvious side effects[58, 59]. It has also been reported that high $ZnSO_4$ concentrations are correlated with enhanced sperm parameters, including sperm count, motility, and normal morphology [60-62] while lower content of $ZnSO_4$ has been observed in the seminal plasma of infertile subjects with poor sperm production and poor sperm motility[63].There are several mechanisms by which $ZnSO_4$ might interfere with sperm function. First, $ZnSO_4$ is a cofactor for several hundred metalloenzymes, particularly the enzymes responsible for protein synthesis [64, 65]. It influences phospholipases, thus modulating the stability of biological membranes. It has been suggested that the removal of $ZnSO_4$ from the sperm cell surface destabilizes the plasma membrane, playing an important role in preparation for the completion of capacitation and the acrosome reaction. Some studies have reported that $ZnSO_4$ supplementation can also improve the synthesis of metallothioneins which protect sperm against damage [66]. It has also been suggested that $ZnSO_4$ in seminal plasma is involved in maintaining the stability of sperm chromatin [67] and may also exert an*in vitro* e□ect on oxidative changes in human semen as it is considered a scavenger of excessive O_2 production by defective spermatozoa and/or leukocytes after ejaculation [68].

CONCLUSION

This study assessed the reproductive function in rats exposed to BLCO and treated with NAC and ZnSO4. It demonstrated the ability of NAC and ZnSO4, administered at different doses, to improve and restore reproductive function in these BLCO-exposed rats. The results indicate that NAC and ZnSO4 are powerful antioxidants that can counteract the harmful effects of oxidative damage caused by ROS generated during BLCO exposure. Additionally, the study showed that

NAC and ZnSO4 protected the testes, preventing reproductive alterations linked to BLCO exposure

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