

Testicular morphology and epididymal sperm reserves of male rats following the withdrawal of Nigerian Qua Iboe Brent crude oil

Udensi M. Igwebuike^{1,4*}, Reginald I. Obidike², Njoku U. Njoku¹,
and Shodeinde V. O. Shoyinka³

¹Department of Veterinary Anatomy, University of Nigeria, Nsukka, Nigeria

²Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria

³Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

⁴Abdus Salam International Centre for Theoretical Physics, Trieste, Italy

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ABSTRACT

The reversibility of crude oil-induced male reproductive abnormalities was studied in fifty-six male Sprague-Dawley rats. Exposure to crude oil was achieved by oral administration of increasing doses (165mg/kg body mass, 330mg/kg body mass, and 660mg/kg body mass) of Nigerian Qua Iboe Brent crude oil to male rats every 48 hours for 4 weeks. Thereafter, the crude oil was withdrawn from some of the rats for a period of 8 weeks. The results showed significant reduction in the cauda epididymal sperm reserves of the rats that received the crude oil ($P < 0.05$) and the rats from which the crude oil was withdrawn ($P < 0.05$), relative to the control group. The testes of the rats that received crude oil showed reduced spermatogenic activity in the seminiferous tubules, as well as hyperaemia and oedema of the interstices. The testes of the rats from which crude oil was withdrawn showed evidence of recovery and the restoration of active spermatogenesis in the seminiferous tubules. The indication is that the withdrawal of Nigerian Qua Iboe Brent crude oil from male rats over a period of 8 weeks reversed the testicular pathology, but not the cauda epididymal oligospermia associated with this environmental pollutant.

Key words: crude oil, oestrogen-mimicking chemicals, cauda epididymal sperm reserve, testes, rats

Introduction

Health risks associated with exposure to environmental pollutants have received increasing attention in recent times. Previous studies (ORISAKWE et al., 2004; OBIDIKE et

*Corresponding author:

Dr. Udensi Maduabuchi Igwebuike, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria, Phone: +234 8038 726150; Fax: +234 4277 0644; E-mail: abuchi2002@yahoo.com

al., 2007) have demonstrated that the exposure of male rats to crude oil adversely affects the morphology of the testes, leading to disruption of spermatogenesis in the seminiferous tubules and oligospermia in the cauda epididymides. These findings indicate that crude oil may pose serious reproductive risks to animals and humans, especially in areas where continual oil spillage occurs. The chemical composition of crude oil (hydrocarbons) is capable of mimicking the inherent actions of reproductive hormones, and hence has the ability to disrupt the neuroendocrine system, or more directly, the functions of the gonads (VEERAMACHANENI, 2000). Indeed, some polycyclic aromatic hydrocarbons and other compounds derived from petroleum products have been listed as xenoestrogenic substances (DAVIS et al., 1993). Oestrogen-mimicking chemicals may bind to intracellular receptor proteins for steroid hormones (KORACH et al., 1987), and evoke hormonal effects in animals (GRAY et al., 1989) and humans (GUZELIAN, 1982). These chemicals can act as hormone agonists or antagonists (COLBORN et al., 1993). Crude oil pollution at drilling sites and oil spills on lands used for agricultural purposes, and petroleum or diesel contaminated waste constitute a serious occupational public health risk, to which terrestrial wildlife and livestock are also exposed. Furthermore, crude oil is used by certain local populations in Nigeria as a folk medicine in the treatment of gastrointestinal ailments (ORISAKWE et al., 2000). It has been suggested that the ingestion of crude oil may be responsible for some cases of male infertility in Nigeria (ORISAKWE et al., 2004).

The reversibility or otherwise of these cases of male infertility is of great concern to the public, health workers and researchers. The present study seeks to investigate whether or not withdrawal of Nigerian Qua Iboe Brent crude oil from exposed groups of male rats reverses the reproductive risks associated with this contaminant.

Materials and methods

Experimental animals. The fifty-six male Sprague-Dawley rats used for this study were obtained from the animal house of the Department of Veterinary Anatomy, University of Nigeria Nsukka. The rats weighed between 200 and 250 grams. They were housed in fly-proof metal aluminum cages and fed commercially prepared feed ad libitum. Drinking water was provided. The rats were kept for a 2-week acclimatization period before the commencement of the experiment. They were randomly assigned to 4 groups. The control group (n = 8) received no crude oil, while rats in the test groups (n = 16 per group) were given oral doses of Nigerian Qua Iboe Brent crude oil every 48 hours for 4 weeks, using a rat gavage needle. On each treatment day, the low dose group received

165 mg/kg body mass of crude oil per rat, the medium dose group received 330mg/kg body mass of crude oil per rat, and the high dose group received 660mg/kg body mass of crude oil per rat. These doses fall within the range of doses used by some local populations in Nigeria as folk medicine (ORISAKWE et al., 2004).

At the end of 4 weeks of treatment with crude oil, 24 rats (8 from each of the three treatment groups) were randomly selected. The selected rats and all the rats in the control group were put down by cervical dislocation. Crude oil was withdrawn from the remaining rats in the treatment groups (n = 8 per group) for a period of 8 weeks.

These rats constituted the crude oil withdrawn groups. At the end of the 8 week withdrawal period, these rats were also put down by cervical dislocation.

Quantitative measurements. Following death, the epididymides of rats in the control and test groups were dissected and extraneous tissues trimmed. The epididymides were weighed and put in bijour bottles containing phosphate-buffered saline, pH 6.8. The cauda epididymal sperm reserves were determined using the standard hemocytometric method (AMANN and ALMQUIST, 1961).

Histological preparations. Testes were dissected from rats in the control and test groups. These were fixed by immersion in Bouin's fluid for 48 hours. Later, they were dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. The 5 µm thick sections were cut, mounted on glass slides, and stained with hematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd. 1999-2004).

Statistical analysis. Means and standard errors of cauda epididymal sperm reserves were calculated for the control and each treatment group. The data were statistically analyzed using ANOVA (SPSS 9.0 Statistical Package of SPSS Inc. USA).

Results

Cauda epididymal sperm reserves. Table 1 shows the data on the cauda epididymal sperm reserves in the control, low dose and low dose withdrawn groups. The cauda epididymal sperm reserve of the low dose group was significantly reduced relative to the control group ($P < 0.05$). The cauda epididymal sperm reserve of the group from which crude oil was withdrawn did not differ significantly from that of the control group, neither did it differ from the low dose group ($P > 0.05$). Data on the cauda epididymal sperm reserves of the control, medium dose and medium dose withdrawn groups are presented in Table 2. The cauda epididymal sperm reserve was significantly reduced by the medium dose when compared to control ($P < 0.01$). Withdrawal of crude oil resulted in a significant increase in cauda epididymal sperm reserve relative to the medium dose group ($P < 0.05$), but the cauda epididymal sperm reserve of the crude oil withdrawn group remained significantly lower than that of the control group ($P < 0.05$).

Table 3. presents the data regarding the cauda epididymal sperm reserves of the control, high dose and high dose withdrawn groups. The cauda epididymal sperm reserve was significantly reduced by the high dose relative to the control ($P < 0.05$). Following the withdrawal of crude oil, the cauda epididymal sperm reserve remained significantly

reduced when compared to the control ($P < 0.05$), and there was no significant difference between the cauda epididymal sperm reserve of the high dose and crude oil withdrawn groups ($P > 0.05$).

Table 1. Cauda epididymal sperm reserves of control, low dose and low dose-withdrawn groups

Parameter	Control	Low dose (165 mg/kg b.m.)	Low dose + withdrawal	P level
Cauda epididymal sperm reserves (10^6)	268.68 ± 15.74 ^a	228.59 ± 9.29 ^b	253.86 ± 3.13 ^{ab}	ab: $P < 0.05$

Values are presented as mean ± SE. Different superscripts in a row indicate significant variation at the specified level of probability

Table 2. Cauda epididymal sperm reserves of control, medium dose and medium dose-withdrawn groups

Parameter	Control	Medium dose (330 mg/kg b. m.)	Medium dose + withdrawal	P Level
Cauda epididymal sperm reserves (10^6)	268.68 ± 15.74 ^a	71.76 ± 5.28 ^b	179.81 ± 14.04 ^c	ab: $P < 0.01$ ac: $P < 0.05$ bc: $P < 0.05$

Values are presented as mean ± SE. Different superscripts in a row indicate significant variation at the specified level of probability

Table 3. Cauda epididymal sperm reserves of control, high dose and high dose-withdrawn groups

Parameter	Control	High dose (660 mg/kg b.m.)	High dose + withdrawal	P Level
Cauda epididymal sperm reserves (10^6)	268.68 ± 15.74 ^a	59.73 ± 4.89 ^b	117.14 ± 46.90 ^b	ab: $P < 0.05$

Values are presented as mean ± SE. Different superscripts in a row indicate significant variation at the specified level of probability

Histology. The testes of rats in the control group showed normal seminiferous tubules and interstices (Fig. 1). The morphology of the testes of the rats that consumed crude oil was characterized by severe reduction in the activity of spermatogenic cells of the seminiferous tubules, as well as, hyperaemia and exudation of fluid into the interstices (Fig. 2). Similar testicular pathology was observed in all the crude oil exposed groups, and the magnitude of the pathology was dose-dependent.

The germinal epithelium of the seminiferous tubules of rats from which the crude oil was withdrawn were active in spermatogenesis, and the interstices were normal (Fig. 3).

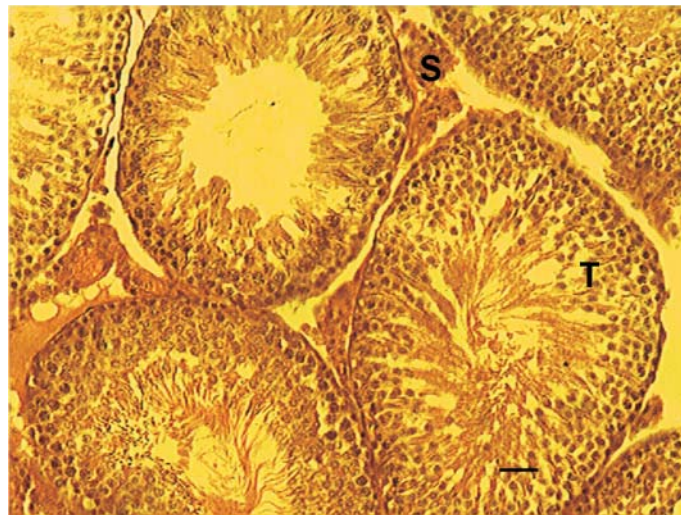


Fig. 1. Testis of control rat showing seminiferous tubules (T) with normal spermatogenic cells. Note interstitial spaces (S) containing Leydig cells. Scale bar = 40 μ m.

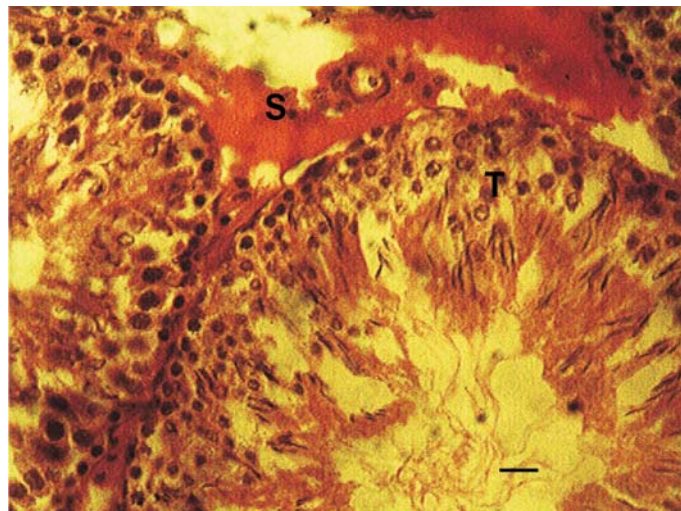


Fig. 2. Testis of rat treated with 330mg/kg of crude oil showing spermatogenic cells of poorly active seminiferous tubules (T). Note hyperaemic and oedematous interstitial spaces (S). Scale bar = 20 μ m.

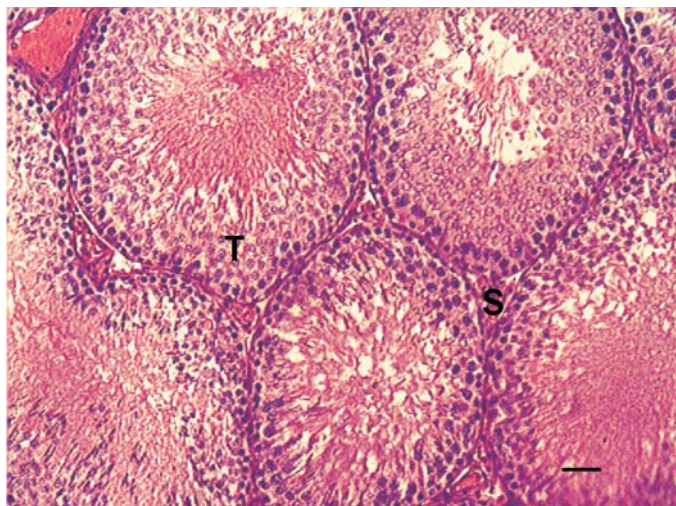


Fig. 3. Testis of rat from which 330mg/kg of crude oil was withdrawn showing spermatogenic cells of active seminiferous tubules (T). Note normal interstitial spaces (S). Scale bar = 40 μ m.

Discussion

Oestrogens influence the neuroendocrine system (FLINCH et al., 1984), and exert biological actions on the reproductive tract and other target organs. These oestrogen responsive sites are highly sensitive and it is possible that exposure to slightly active compounds may alter the natural hormonal balance. In the present study, exposure to Nigerian Qua Iboe Brent crude oil gave rise to a significant reduction in the cauda epididymal sperm reserves of male rats at the low, medium and high treatment doses. Furthermore, the morphology of the testes was adversely affected by the crude oil, and was characterized by reduced spermatogenic activity and the presence of interstitial exudates. These observations agree with our previous report (OBIDIKE et al., 2007). Withdrawal of Nigerian Qua Iboe Brent crude oil from male rats for a period of 8 weeks did not significantly ameliorate crude oil-induced cauda epididymal oligospermia. This effect was more pronounced in the rats exposed to the medium and high doses of crude oil prior to withdrawal.

Although experimental studies have shown that permanent changes occur in the brain (BRAUER et al., 1978), vaginal epithelium (ADLER and NELSON, 1988) and prostate gland (DEKLERK et al., 1979) following the administration of oestrogenic chemicals to adult animals, the testicular morphology of the rats from which Nigerian Qua Iboe Brent crude oil was withdrawn showed evidence of recovery and the restoration of active

spermatogenesis in the seminiferous tubules. This finding supports the general assumption that, after maturity, exposure to endocrine disruptors does not permanently alter the functioning of hormone-responsive tissues. The significantly reduced cauda epididymal sperm counts in these rats may be an indication that the spermatozoa produced in the recovering testes did not sufficiently re-populate the cauda epididymides during the 8 week withdrawal period so as to restore the epididymal sperm counts to normal values. Thus, the cauda epididymal sperm counts of the rats from which the medium and high doses of crude oil were withdrawn remained significantly lower than control values.

In conclusion, our study demonstrated that the withdrawal of Nigerian Qua Iboe Brent crude oil from male rats over a period of 8 weeks reversed testicular pathology, but not the cauda epididymal oligospermia associated with this environmental pollutant.

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SAŽETAK

Reverzibilnost reprodukcijских poremećaja uzrokovanih sirovom naftom istraživana je na 56 štakora Sprague-Dawley. Peroralno su štakorima bile davane sve veće doze (165 mg/kg tjelesne mase, 330 mg/kg tjelesne mase i 660 mg/kg tjelesne mase) nigerijske Qua Iboe Brent sirove nafte svakih 48 sati tijekom četiri tjedna. Nakon toga neki od štakora više nisu dobivali sirovu naftu u razdoblju od osam tjedana. Rezultati su pokazali značajno smanjenje rezervi sperme u epididimisu štakora koji su dobivali sirovu naftu ($P < 0,05$) i u štakora kojima se prestalo davati sirovu naftu ($P < 0,05$) u odnosu na kontrolnu skupinu. Testisi štakora koji su dobivali sirovu naftu pokazivali su smanjenu sposobnost spermatogeneze u sjemenskim tubulima, a dokazana je bila hiperemija i edem intersticija. Nakon prestanka davanja sirove nafte testisi su se oporavili te je ponovo došlo do aktivne spermatogeneze u seminiferim tubulima. Aktivnost testisa vratila se na normalu osam tjedana nakon prestanka davanja nigerijske sirove nafte Qua Iboe Brent, ali ne i oligospermija repa epididimisa povezana sa zagađenjem okoliša.

Ključne riječi: sirova nafta, kemikalije slične estrogenima, pričuva sperme, testis, štakor
