Original Article

Hormonal assays following oral administration of bonny light crude oil on male wistar rats

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Abstract

Introduction: Crude oil is composed of a wide range of chemicals and its hydrocarbon content differs widely depending on the location and source. Bonny light crude oil (BLCO) is Nigeria's marker crude oil with a low sulfur content and low corrosive property. The effect of BLCO on the serum levels of reproductive hormones was evaluated in male Wistar rats. **Methodology:** Seventy-five (75) male Wistar rats weighing between 150 and 200 g were divided into five groups A, B, C, D and E consisting of 15 animals. Groups A-D served as the experimental groups and Group E served as the control that received normal saline. 0.5, 1.5, 2.5 and 3.5 ml/kg bw of BLCO was administered to the experimental animals in Groups A, B, C and D respectively via oral gastric intubation once on alternate days for 60 days. The animals were sacrificed and blood was collected via cardiac puncture, centrifuged and the serum was collected for hormonal assay using the enzyme-linked immuno-absorbent assay (ELISA) method. **Results:** There were significant ($P < 0.05$) higher serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the treatment groups particularly in the 2.5 and 3.5 ml/kg bw groups when compared with the control. There was also a significant ($P < 0.05$) decrease in the serum testosterone hormone (TH) level in the treatment groups. The effects were observed to be dose dependent. **Conclusion:** BLCO has significant effects on the serum levels of LH, FSH and TH of male Wistar rats and the effect may be at the level of the testis.

Key words: Bonny light crude oil, follicle stimulating hormone, luteinizing hormone, testosterone, Wistar rats

INTRODUCTION

Crude oil is a complex mixture containing thousands of different chemicals. However the major chemical composition of crude oil is hydrocarbon that tends to vary widely by location and source [Briggs *et al*., 1996]. Crude oil and petroleum products share certain toxic characteristics and are toxic to biological life [Walkinson and Holt, 1987]. Bonny light crude oil (BLCO) is a high grade of Nigerian crude oil with high API gravity (low specific gravity) produced in the Niger Delta Basin and named after the city of Bonny [Lambert and Shaw, 1982].

Industrial development through exploitation and exploration of crude oil or total petroleum hydrocarbon (TPH) has introduced into the ecosystem substances that are potentially toxic to life and environment [Dede, and Kagbo, 2002]. Since the discovery of oil in Nigeria in 1958, the country has suffered the negative environmental consequences of oil development. Oil spills in the Niger delta has been a regular occurrence, and the resultant degradation has caused significant tension between the people living in that region and multinational companies

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operating there. Oil spillage during production, exploration and discharge from storage facilities and refineries and bursting of pipelines is one of the fundamental sources of hazard and pollution in the oil producing communities of Nigeria [Lambert and Shaw, 1982; Akpofure *et al*.; 2000, Peters, 1993, Okereke and Ezeanyina, 1987].

The devastating consequences of spills of this crude in the Niger delta region with its eventual hazards on both aerial and terrestrial environs tantamount to an irreversible chain effect on the biodiversity and human safety [Akpofure *et al*.; 2000]. The epidemiological data and result of toxicity studies in experimental animals consistently report there is significant health risk due to prolonged exposure to petroleum products [Didia *et al*.; 2003, Dede and Kagbo, 2002, Dede *et al*.; 2002, Eyong, 2000].

Several polyaromatic hydrocarbons (PAHs) have caused tumours in laboratory animals by inhalations, by oral ingestion or by skin contact. Neurotoxic effects have been detected in workers exposed to petroleum solvents [Environmental Health Criteria, 1982]. Crude oil is considered to be a developmental toxicant and as such have the potential to induce adverse effects such as pregnancy terminations and malformations [Feuston and Hamilton 1997, Feuston and Mackerer 1996, Fischer *et al*.; 2007, 2006, 2005] sterility in offspring and testicular changes including wasting with lack of sperm [Obidike *et al*.,1989; Orisakwe *et al*., 2004]. Benzene a component of crude oil is one of the well-established genotoxics [Harty and Chaman, 1990]. The toxic properties of petroleum hydrocarbons include a variety of mutagenic responses and tumour formation [McKee *et al*.; 1994, Pasquini *et al*.; 1989].

The increasing trend of male reproductive impairment observed in some countries has been associated with possible exposure to chemicals that could interfere with endocrine homeostasis (endocrine disrupting chemicals EDC) [Bergstrom *et al*.; 1996, Bergmann *et al*.; 1999, Moller 2001, Moline 2000]. Crude oil is composed of a wide range of chemicals [Briggs *et al*., 1996] and these chemicals are capable of mimicking the inherent actions of reproductive hormones and hence have the ability to disrupt the neuroendocrine system [Colborn *et al*.; 1998].

This research was thus done to evaluate the effect of ingestion of BLCO on the serum levels of pituitary gonadal hormones and its effect on fertility potential in male Wistar rats.

MATERIALS AND METHODS

In this original and experimental research, 75 male Wistar rats weighing between 150 and 200 g were left to acclimatize for 3 weeks in the animal house of the Department of Human Anatomy, University of Calabar, Calabar. Approval was obtained from the Ethics Committee of the University, care and management of the animals was strictly adhered to. Feeds and water were allowed *ad libitum*. The animals were grouped into five groups consisting of 15 experimental animals and 15 control animals. BLCO used for this study was obtained from Shell Petroleum Development Company (SPDC), with permission from the Department of Petroleum Resources N.N.P.C Lagos.

Experimental animals in groups A, B, C and D received 0.5, 1.5, 2.5 and 3.5 ml/kg bw of BLCO respectively, while the control animals received normal saline. The dose of BLCO was determined from previous research [Fischer *et al*.; 2007; 2006; 2005]. The BLCO was administered via oral gastric intubation once on alternate days for 60 days.

The animals were sacrificed by chloroform anaesthesia to avoid raised serum levels of hormone and blood was collected via cardiac puncture from the left ventricle of the heart. Clotted blood was then centrifuged using 80-2 electric table centrifuge at 300 rpm for 10 minutes to recover serum from clotted cells, serum was separated with sterile syringe and stored frozen for hormonal assay. Luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone hormone (TH) in the serum were determined using the enzyme-linked immuno-absorbent assay (ELISA) method with Microwell's kit.

Statistical Analysis

The results were analysed using analysis of variance (ANOVA) using the SPSS statistical program and *post hoc* test to compare results of the experimental and control groups. P values < 0.05 were reported to be statistically significant.

RESULTS

Luteinizing Hormone

There was a significant increase at $P<0.05$ in serum LH level of groups A, B, C and D treated with 0.5, 1.5, 2.5 and 3.5 ml/kg bw of BLCO respectively when compared with the control group given normal saline. A: 1.25 ± 0.05, B: 1.80 ± 0.50, C: 2.20 ± 0.40, D: 4.0 ± 0.30 of the BLCO-treated rats compared to the control group E: 0.20 ± 0.56.

Follicle Stimulating Hormone

There was significant increase at $P<0.05$ in serum FSH level of groups A: 0.30 ± 0.07, B: 0.40 ± 0.30, C: 3.10 ± 2.30, D: 4.50 ± 0.70 of the BLCO-treated rats compared to the control group E: 0.02 ± 0.0.

Table 1: The serum hormonal levels of LH, FSH and TH in the control and 0.5, 1.5, 2.5 and 3.5 ml/kg BLCO groups

Hormones	A	B	C	D	E
	0.5 ml/kg of BLCO	1.5 ml/kg of BLCO	2.5 ml/kg of BLCO	3.5 ml/kg of BLCO	Control groups
LH	1.25±0.05**	1.80±0.50**	2.20±0.40**	4.00±0.30**	0.20±0.56
FSH µ/ml	0.30±0.07**	0.40±0.30**	3.10±2.30**	4.50±0.70**	0.02±0.0
TH µ/ml	0.43±0.12^{NS}	0.40±0.68^{NS}	0.13±0.06^{NS}	0.03±0.06**	1.10±0.10

n=15 Results are presented as mean±SEM. ^{NS}Not significantly different from the control at *P*<0.05, **Significantly different from the control at *P*<0.05, LH - Luteinizing hormone, FSH - Follicle-stimulating hormone , TH - Testosterone hormone , BLCO - Bonny light crude oil

Testosterone Hormone

Treatment with BLCO caused a decrease in serum TH level in groups A: 0.43 ± 0.12, B: 0.40 ± 0.68, C: 0.13 ± 0.06, D: 0.03 ± 0.06 when compared with the control group E: 1.10 ± 0.10. The decrease was significant in the group D animals at $P < 0.05$.

DISCUSSION

This study investigated the effect of BLCO on the serum levels of LH, FSH and TH. TH, the major product of the testes, is a primary inhibitor of LH secretion in males. FSH levels are the most important endocrine parameters to evaluate testicular function [Bergmann *et al*.; 1999]. Measurement of FSH and LH can establish the point of defect along the hypothalamic – pituitary – testicular axis. Increased serum FSH and LH shows that the defect is in the hypothalamus or pituitary gland. LH and FSH both bind to specific receptors on the Leydig cells and Sertoli cells respectively within the testis. LH affects spermatogenesis indirectly in that it stimulates androgenous TH production. Testosterone in turn inhibits LH secretion while FSH targets sertoli cells. TH and FSH are hormones that are directed at the seminiferous tubules epithelium. The mechanism of feedback control of FSH is regulated by inhibin, Sertoli cell product. A decrease in spermatogenesis is accompanied by decreased production of inhibin and this reduction in negative feedback is associated with reciprocal elevation of FSH levels [Ashiru and Blake, 1979]. A normal level of these hormones is indicative of normal sexual processes in the males.

In this study, LH and FSH levels were significantly higher ($P < 0.05$) in 0.5 ml/kg, 1.5 ml/kg, 2.5 ml/kg and 3.5 ml/kg BLCO-treated groups while TH levels were significantly lower ($P < 0.05$) in groups treated with 2.5 ml/kg and 3.5 ml/kg of BLCO. This may suggest that BLCO may have adversely affected Leydig and Sertoli cells inhibiting spermatogenesis which may have resulted in subsequent inhibition of TH synthesis resulting in stimulation of LH and FSH secretion. This study is in line with Obidike *et al*.[2007] who reported the presence of interstitial exudates, degeneration and necrosis of spermatogenic and interstitial (Leydig) cells in the testis of rats exposed to Nigerian Qua Iboe Brent crude oil. Orisakwe *et al*.[2004] reported reduction in epididymal sperm count, slight or severe degeneration or even complete absence of seminiferous tubules and necrosis of cells following administration of BLCO dissolved in drinking water to albino Wistar rats. However, TH levels were not significantly different ($P < 0.05$) in groups treated with 0.5 ml/kg and 1.5 ml/kg of BLCO compared with the control.

In conclusion, administration of BLCO showed significant alterations in the hormonal profiles seen in the results of the serum levels of LH, FSH and TH, implying that BLCO possibly adversely affects the reproductive hormones secretion and the effect may be at the level of the testis.

REFERENCES

1. Akpofure E.A., Efere M.L., Prosper A. (2000). Integrated grass root post-impact assessment of acute damaging effects of continous oil spill in Niger Delta (January 1998-2000). Oil spill Intelligence Report. 1-9.
2. Ashiru O, Blake E. (1979). Stimulation of androgenous FSH release during oestrus by administration of exogenous FSH or LH at proestrus in the Phenobarbital-blocked rat. Endocrinology 105:1162-7.
3. Bergmann M., Belire H.M., Niechlag E. (1999). Serum FSH and testicular morphology in male infertility. Clin Endocrinol 40: 133-6.
4. Bergstrom R., Adam H.O., Mohner M., Zatonski W., Storm H., Ekbom A, *et al*. (1996). Increase in testicular cancer in six European countries. A birth cohort phenomenom. J Natl Cancer Inst 88:727-33.
5. Briggs K.T., Yoshida S.H., Gershwin M.E. (1996). The influence of petrochemicals and stress on the immune system of seabirds. Regul Toxicol Pharmacol 23:145-55.
6. Colborn T., Vom Saal F.S., Soto A.M. (1993). Developmental effects of endocrine disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378-84.
7. Dede E.B., Kagbo H.D. (2002). A study on the acute toxicological effects of commercial diesel fuel in Nigeria on rats (Ratus ratus) using haematological parameters. J Appl Sci EnvironManage 5-9.
8. Dede E.B., Igboh N.M., Ayalogu O.A. (2002). Chronic study of the effect of crude petroleum (Bonny Light), kerosine and gasoline on rats using haematological parameters. J Appl Sci Environ Manage 6 (1):60-3.
9. Didia B.L., Dede E.B., Dapper D.V. (2003). Effects of crude oil contaminated water on haematocrit and histopathology of Guinea pig: Animal mode for investigating crude oil pollution. J Exp Clin Anat. 2 (2): 6-11.
10. Environmental Health Criteria (EHC) (1982) Selected petroleum products. United Nations Environment programme. World Health Organisation Publication, Geneva. 20:5-118.

11. Eyong E.U. (2000). Biochemical and toxicological implication of ingestion by rats of shelfish exposed to crude oil polluted. Ph. D. thesis, University of Calabar, Nigeria.
12. Feuston M.H., Hamilton C.E. (1997). Developmental toxicity study in rats exposed dermally to clarified slurry oil for a limited period of gestation. J Toxicol Environ Health 49 (2):207-20.
13. Feuston M.H., Mackerer C.R. (1996). Chronic Dermal Studies of petroleum streams in mice. Fundam Appl Toxicol 301 (1): 47-54.
14. Fischer V.A., Akpuaka F.C., Ngokere A.A. (2005). Effects of Bonny Light Crude Oil on the histology of cerebral cortex in fetuses of wistar rats. J Exp Clin Anat 4 (1):9-11.
15. Fischer V.A., Anibeze C.I., Igiri A.O., Ekanem T.B., Mesembe O.E., Fischer C.E. (2007). Effect of maternal administration of Bonny Light Crude Oil on brain dimensions of wistar rat fetuses. Glob J Pure Appl Sci 13 (1):95-7.
16. Fischer V.A., Anibeze C.I., Igiri A.O., Eyong E.O., Mesembe O.E., Fischer C.E. (2006). Effects of Bonny Light Crude Oil on Morphology of litters of wistar rats. Glob J Med Sci 5 (1):51-3.
17. Harty W.R., Chaman E.V. (1990). A toxicological assessment of unleaded gasoline contamination of drinking water in petroleum contaminated soils. In: Kosteck P.T., Calabrese E.J., editors. Vol. 3. Lewis Publication Inc.:324-40.
18. Lambert A.A., Shaw H. (1982). Environmental effects of crude oil spillage. Glob J Pure Appl Sci 1:85-90.
19. McKee R.H., Amoruso M.A., Freeman J.J. (1994). Evaluation of the genetic toxicity of middle distillate fuels. Environ Molecular Mutagen 23:234-8.
20. Moline J.M., Golden A.L., Bar-Chama N., Smith E., Rauch M.E., Chapin R.E., *e al*. (2000). Exposure to hazardous substances and male reproductive health. A research framework. Environ Health Perspect 108 (9):803-13.
21. Moller H. (2001). Trends in incidence of testicular cancer and prostate cancer in Denmark. Hum Reprod 16:1007-11.
22. Obidike R., Igwebuike U.M., Shoyinka S.V. (1987). Testicular morphology and epididymal sperm reserves of male rats exposed to Nigerian Qua Iboe Brent crude oil. J Pure Appl Sci 1:85-90.
23. Okereke C., Ezeanyina I. (1987). Environmental side effects of crude oil spillage. Glob J Pure Appl Sci 1: 85-90.
24. Orisakwe O.F., Afonne O.J., Nwobode E., Asomugha L., Dioka C.E. (2004). Testicular toxicity of Nigeria's bonny light crude oil in male albino rats. Reprod Toxicol 18: 439-42.
25. Pasquini R., Taningher M., Monarca S., Pala M., Angeli G. (1989). Chemical composition and genotoxicity of petroleum derivatives collected in two working environments. J Toxicol Environ Health 27 (2):225-38.
26. Peters S.W. (1993). Crises in our environment. Nigeria Environmental Education and Management. University of Calabar Press, Calabar. p. 126-48.
27. Walkinson R.H., Holt M.S. (1987). Biodegradability of produced water effluents; medicinal problems in the off-shore oil industry. Proc. Int. conference of institute of petroleum microbiology committee. Amerdeem. John Wiley and Sons, USA. p. 165-74.

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