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Full Length Research Paper

Spermatotoxic impact of bonny light crude oil (BLCO) ingestion on adult male Swiss albino mice

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Increasing concern has been expressed about the possible declining trend in the sperm quality and sperm count of man as a result of exposure to environmental estrogenic agents in the past few years now. There is a general paucity of knowledge of BLCO ingestion on the reproductive effect. Hence, we aim to evaluate the impact of sub-lethal dose of BLCO ingestion on semen parameters of adult male mice. Initial acute toxicity study was carried out to determine the lethal dose of BLCO, which was calculated to be 37.4 mg/Kg body wt. A sub-lethal dose of 20 mg/Kg bwt /day of BLCO were then given to 8 male mice in the experimental group. While, the control group of 7 animals received equal volume of 0.9% normal saline via oral garvage for 2 weeks. Data were analysed using SPSS 12 statistical software with $P < 0.05$ considered statistically significant. There was a significant ($P < 0.05$) weight gain in the treated group with a significant ($P < 0.05$) reduction in sperm motility in the treated compared with control. The sperm density of treated and control were 14.5 x 10^6 /ml and 20.5 x 10^6 /ml respectively. However, there were also no significant difference in the relative testicular weight and sperm density of treated from that of the control respectively. Thus, it was concluded that BLCO ingestion is spermatotoxic in the adult male Swiss mice

Key words: BLCO, sub-lethal dose, sperm density, motility, adult male mice.

INTRODUCTION

Increasing concern about the possible declining trend in the sperm count of man and wildlife animals over the past decades as a result of exposure to various environmental estrogenic agents (aromatic hydrocarbons) has been expressed recently (Izegbu et al., 2005; Lyons et al., 1999; Shittu et al., 2007; Shittu et al., 2008).

The scarcity of petroleum product in Nigeria sometimes in the years gone by and the booming "black market" dealing in refined crude oil has led to the situation where-by petroleum product has continually been exposed to the human system-via orogastric passage or accidental ingestion especially by the use of rubber hoses to suck fuel from car tanks into various categories of containers.

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However, limited exposure to crude oil may occur during drilling, transporting and refining. Accidental spill-age accounts for a more serious exposure to crude oil by wild life and humans. An example of such spillage occu-rred in Nigeria in November 2002 (WWF, 2002).

Many of the people who live in the oil rich areas are exposed to water from streams and ponds that have been polluted by oil spillage at one time or the other. This water is used for domestic activities such as drinking, cooking and washing by rural dwellers in the various oil rich- areas of the Niger Delta in South-South Nigeria. However, majority of the people in the communities ingest crude oil either directly as curative agents for anti poisoning (snake venom antidotes), anti-convulsion, treatment of skin infection e.g. scabies or indirectly by eating marine animals found in surrounding coastal waters as source of protein (Dede et al., 2002).

Other studies have shown that cytotoxic and biochemi-

cal derangements are associated with ingestion of marine animals in polluted areas (Eyong, 2000; Nwaokwoala and George, 2000). Moreover, brain damage such as cerebral cortex malformations were also been observed in fetuses of pregnant rats exposed to bonny light crude oil in a recent study (Fischer et al., 2005).

There is a general paucity of knowledge on the effect of BLCO on the male reproductive system in mammals including man. We aim to carry out an acute reproductive toxicity study on adult male Swiss albino rats using histomorphometric and seminal studies.

MATERIAL AND METHODS

Animals used

40 adult mice of both sexes mice weighing between 17 - 30 g procured from the animal house of the Nigeria institute of medical research, Yaba, Lagos were used for this experiment. The mice were kept in the animal control room of Lagos state university, college of medicine, Ikeja to acclimatize for 2 weeks before the experiment commenced.

The mice were fed with a standard chows/pellets (Pfizer Nigeria Limited, Ikeja), given water *ad libitum* and maintained under standard conditions. The room was well ventilated with a temperature range of 25 - 27^{o}C under day/night 12 - 12 h photoperiodicity. Animal care with control rule and regulation of the institution was considered during the whole experiments.

The body weights of the animals were taken all through the experimental period and prior to animal sacrifice.

Determination of lethal dose/ acute toxicity study

20 animals were divided randomly into 5 groups of 5 mice each and fasted 24 h prior to treatment with BLCO. The Bonny light crude oil (BLCO) was obtained from a master student in the biological science faculty, university of Lagos and were administered orally by garvage to animals in the different groups of animals- A, B, C, D, E group in the following dose range: 20 mg/kg bwt, 30 mg/Kg bwt, 40 mg/Kg bwt, 50 mg/Kg bwt and 60 mg/Kg bwt respectively. After treatment, water and foods were given *ad libitum*.

The animals were closely observed for signs of toxicity over a period of 96 h, the number of death recorded every 24 h were taken. The survivors were monitored for a week after which they were discarded.

The sliding method was used in the determination of the LD_{50}, which was then extrapolated through graph plotting.

Reproductive study

2 different groups A and B were randomly assigned 8 and 7 mice respectively, such that, the treated group A received a sub-lethal dose of 20 mg/Kg bwt of BLCO via oral garvage based on the lethality dose obtained above. The control group B also received equal volume of normal saline (0.9%) orally daily for 14 days.

The BLCO was administered to the animals at the beginning of the experiments as start dose after a 24 h fasting and the animal were then observed for 2 weeks to allow for a complete epididymal sperm transport after treatment, because it takes about 10 – 14 days for newly formed sperm cell to travel through the epididymis (Consetino et al., 1990).

Animal sacrifice

At the termination of the study, the animals were sacrificed by anesthesia as previously described in our study (Shittu et al., 2007).

Organ harvest and tissue processing for light microscopy

Both the testis and epididymes were carefully dissected out, trimmed of all fat and blotted dry to remove any blood. Their weights were noted and volume measured by water displacement with the aid of a 10 ml measuring cylinder as earlier described by Shittu
(2006).

Histological preparation of the fixed tissues in 10% formal saline were carried out and serial sections of 5 µm thickness of processed tissues were stained with H & E stains as described in our previous study (Shittu et al., 2007; Shittu et al., 2008).

Cauda sperm forward motility

After anesthetizing the rat, epididymis was exposed by scrotal incision and spermatozoa were expressed out by cutting the distal end of the cauda epididymidal tubule. Spermatozoa with epididymal fluid was diluted with physiological saline and placed on a thin glass slide and forward motility (rate and percentage) of 100 spermatozoa /mouse was observed under microscope at X 400 magnification as previously described in Shittu (2006).

Sperm count

Spermatozoa were counted following the method described in previous studies (Shittu, 2006; Shittu et al., 2007). Sperm suspendsions were placed on both sides of Neubauer's hemocytometer and allowed to settle by keeping in a humid chamber (wet) for 1 h. The number of spermatozoa in the appropriate squares of the new improved neuber haemocytometer was counted under the microscope at X100 magnification.

Statistical analysis

The weight data were expressed in Mean $\pm$ S.D (Standard deviation) while other data were expressed as Mean ± S.E.M (Standard Error of Mean). Statistical analyses were done by using the student t-test and ANOVA where applicable with input into SPSS 12 software microsoft computer (SPSS, Chicago, Illinois). Statistical significance was considered at $P \leq 0.05$.

Ethical statement

The institution ethic and research committee approved the research.

RESULTS

Animal behaviour

In the acute toxicity studies, there were evidences of increased in locomotor activity, restlessness and sniffing followed by the abdominal stretching at the initial following the crude oil ingestion by the animals. However, the animals made attempt to wipe their mouths repeatedly

Table 1. LD_{50} determination of BLOC ingestion in mice.

Time (h)	LD_{50} mg/kg	Coefficient of correlation	Confidence interval
24	55.21	+0.87	53.99 - 56.43
48	40.61	+0.94	39.38 - 41.88
72	37.40	+0.85	36.01- 38.79
96	37.40	+0.85	36.01- 38.79

Table 2. Body weight of animals (grams) in treated and control groups.

Groups	Pre-treatment weight (g)	Post treatment weight (g)	Body weight gain (g)
Control n =7	19.4 ± 2.3	20.4 ± 0.7	2.02 ± 0.7
Crude oil treated n = 8	21.2 ± 1.0	28.2 ± 0.8*	6.6 ± 0.8*

Data expressed as mean ± S.D
* Significant value at P < 0.05.

Table 3. Semen parameters of the animals.

Parameters	Control n = 7	Treated n = 8
Relative Weight of Testis (g/100g bwt)	0.20 ± 0.03	0.28 ± 0.04*
Sperm density (epididymal sperm reserves) (X 10^6/ml)	20.0 ± 1.3	14.5 ± 2.7 (NS)
Sperm motility (%)	70 ± 8.2	40 ± 6.6*

Other data were expressed as mean ± S.E.M,
*Significant value at P< 0.05.

with their fore limbs, which was later followed by decreesed in locomotor activity observed within 24 h of ingestion.

In addition, the animal`s fur was roughened and changed to dirty white colour. Crude oil was equally noticed to have come out of the anus of the animals in less than 24 h. However, none of the animals died within 30 min after treatment.

Moreover, the survivors regained their appetite for food and water after 96 h. The number of deaths was recorded and the LD_{50} calculated at 24 h interval was 55.24, 40.63, 37.40 and 37.40 mg/kg bwt at 24, 48, 72 and 96 h respectively as shown in Table 1.

Many of the animals used for the toxicity study died especially the ones that received 50 mg/kg B.W and above. Nevertheless, there are a few survivals in other groups taking 40 mg/kg and below. The LD_{50} was calculated to be 37.4 mg/kg at 96.

Reproductive study

The body weight gain of the treated animals in the reproductive studies was significantly ($P < 0.05$) higher than that of the control animals as shown in Table 2.

The raw weight of the testis (g) and the epididymal sperm reserves (density) of the treated groups were lower but not significant ($P > 0.05$) when compared to the control respectively.

While the motility of sperm cells was found to be significantly ($P < 0.05$) reduced in the treated group compared to control (Table 3).

The testicular photomicrographs and histopathological findings of the treated animals were not different from that of the control, hence not included and reported in the present study. This may be a result of the short duration of the present study.

DISCUSSION

Only a few studies are however available in the area of reproductive impacts of crude BLCO ingestion on experimental animals including humans after extensive literature search.

The sperm count of the treated mice used in the present study did not show any significant difference ($P > 0.05$) when compared to the control, except for a lower sperm count obtained in the treated animals. This may be due to its toxic impact on the testis as a whole, especially

The polychlorinated - benzenes (PCB) present in the BLCO (Eyong, 2000).

However, the percentage motility of the treated mice was significantly ($p < 0.05$) reduced when compared to that of the control leading to asthenozoospermia in the treated. This is probably due to the reactive oxygen species (ROS) production or oxidative stress damaged

induced by the toxic`s agents in BLCO on the milieu of the epididymes including that of the male reproductive system of the treated mice as a whole. Similar findings were seen in other previously reported studies (Korach, 1994; WWF, 2002).

Few previous studies have equally demonstrated the impact of other slurry oil and crude oils samples containing 3 to 7 poly aromatic hydrocarbons (PAHs) used in human breast cancer assay and that of other constituents such as the polychlorinated-benzenes (PCB) present in the BLCO (Eyong, 2000) on the male reproductive system. However, they were found to be capable of disrupting the normal reproductive physiology involved with spermatogenesis and other endocrine - mediated developments in general (Acaro et al., 2001; Mably et al., 1992; Pflieger-Bruss et al., 1999; Zhang and Qiao, 2004).

Moreover, other similar studies showed that a single oral dose of crude oil was found to cause growth retardation and other physiological changes such as decrease in accessory sex organs weights, altered sexual behaviour and delayed puberty in wild life animals especially the sea nestling gull (*Larus argentatus*) (Mably et al., 1992; Miller et al., 1978).

This is however, contrary to our findings, where a significant ($P < 0.05$) weight gained was observed in the treated mice when compared to the control animals. This may be as a result of the initial effects of the PAH / PCB and the short duration involved in the present study. This was further, corroborated in a similar observation by Zhang and Qiao (2004).

In addition, we found that no significant difference existed between the relative weight of the testis in the treated and control ($P < 0.05$). Though, this was higher in the treated than the control and unlike, the findings of Zhang and Qiao (2004) with increased in body weights and a decreased in the weight of both testes respectively compared to the control.

However, this causal findings may be due to toxic effects of its PCB /PAH contents and possibly the short duration of this study.

Similarly, other study by Korach and co-workers (1994) have also shown that male mice that lacked functional estrogen receptors displayed a wide variety of reproductive dysfunctional problems such as infertility, abnormal spermatogenesis, reduced testis size and decreased sperm motility as supported by Zhang and Qiao (2004).

In addition, the poly aromatic hydrocarbons (PAHs), one of the major constituents of crude oil (Eyong, 2000) was reported to have adverse effects on the male reproductive system such as testicular changes including wasting with lack of sperm cells, disruptions of sex hormones and induced reproductive toxicity in experimental studies (Peakall et al., 1981; Pflieger-Bruss et al., 1999; WWF, 2002).

Moreover, the PCB present in the BLCO is also an example of estrogenic endocrine disruptors (EED) that can cause disruption to both the reproductive and endocrine organs (Zhang and Qiao, 2004). Interestingly, other previous studies on a particular class of EEDs, known as the phytoestrogenic lignans (found in sesame plants for example) having similar structural pattern to the PCB found in BLCO were nonetheless found to be more desirous in enhancing reproductive and spermatogenic activities in rats including man (Shittu, 2006; Shittu et al., 2007; Shittu et al., 2008).

This was further corroborated by the studies of Hess et al., (1997), where estrogens is found to play a critical role in Leydig cell development and regulation of spermatogenic progression in the testes of many vertebrae.

Thus, from the above findings in the present study, it is obvious that BLCO contained certain EED and toxic agents, which can cause disruption of the normal spermatogenic /reproductive potential and may predisposed to male infertility if not checked.

Conclusion

BCLO ingestion is toxic to the male reproductive system probably via direct toxic impact on the testis/epidiymis or through the production of ROS. Moreover, further study is on going to determine if these effects are reversible and to further demonstrate its effects on the hypothalamo-pituitary –testicular axis using hormonal profile and for a longer duration of time.

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REFERENCES

Arcaro KF, Gierthy JF, Mackere CK (2001). Antiestrogenicity of clarified slurry oil and two crude oils in a human breast cancer cell assay. J. of Toxicology and Environmental Health [part A]. 62: 505-521.

Consetino AJ, Pakyz RT, Fried J (1990). Pyrimethamine: An Approach to the Development of a Male Contraceptive. Proc natl Acad Sci USA, Med Sci 87: 1431-1435.

Dede EB, Igboh NM, Ayalogu OA (2002). Chronic study of crude petroleum bonny light, kerosene, and gasoline using hematological parameters. J Applied Sci and Environ Mgt., 6(1): 60-66.

Eyong EU (2000). Biochemical and toxicological implication of ingestion by rats of shellfish exposed to crude oil from polluted water. Ph.D thesis, University of Calabar, Nigeria.

Fischer VA, Akpuaka FC, Ngokere AA (2005). Effects of bonny light crude oil on the histology cerebral cortex in fetuses of Wistar rat. J. Exp. & Clin. Anat., 4(1): 9-11.

Hess RA, Bunick D, Lee KH, Bahr J, Taylor JA, Korach KS, Lubahn DB (1997). A role for oestrogens in the male reproductive system. *Nature*, 390 (6659):509-512.

Izegbu MC, Ojo MO, Shittu LAJ (2005). Clinical –pathological pattern of testicular malignancies in Ilorin, Nigerua-a report of 8 cases. Journal of Cancer research and therapeutics. 1(4): 229-231.

Korach KS (1994). Insights from the study of animals lacking functional estrogen receptor. Science, 266 (5190): 1524-1527.

Lyons RA, Temple JM, Fone DL (1999). Acute health effects of the sea oil spill J.Epidemiol. comm. Health, 53: 306-310.

Mably TA, Bjerke DL, Moore RW (1992). In utero and lactational exposure of male rats to 2,3,7,8 tetrech-lorodibenzo-*p*-dioxin.3. Effects on spermatogenesis and reproductive capability. Toxicol. Appl. Pharmacol. 114 (1): 118-126.

Miller DS, Peakall DB, Kinter WB (1978). Toxicity of crude oil, Sublethal effects in herring gall chicks. Science 199: 315-317.

Nwaokwoala RN, George W (2000) . Recovery of rats from the effects of prolonged exposure to Nigeria crude oil, West Africa society Pharmacol, XXVII conf. Abstr. Tox pp. 20-73.

Peakall DB, Miller DS, Kinter WB (1981). Toxicity of crude oils and their fractions to nestling herring gulls: physiological and biochemical effects, Marine Environ. Res. 20: 13-21.

Pflieger-Bruss S, Hanf V, Behnisch P, Hagenmaier H, Rune GM (1999). Effects of single polychlorinated biphenyls on the morphology of cultured rat seminiferi. Andrologia 31(2): 77-82.

Shittu LAJ (2006). The effect of the aqueous crude leaves extract of *Sesamum radiatum* compared to Mesterolone (proviron) on the adult male Sprague Dawley rats testis and epididymis. MSc Dissertation, Lagos State University, College of Medicine, August.

Shittu LAJ , Bankole MA, Oguntola JA , Ajala O, Shittu RK , Ogundipe OA , Bankole MN, Ahmed T , Ashiru OA. (2007). Sesame Leaves Intake Improve And Increase Epididymal Spermatocytes Reserve In Adult Male Sprague Dawley Rat. Scientific Research and Essays, 2 (8): 319 -324.

Shittu LAJ, Shittu RK, Ajala MO, Bankole MA, Benebo AS, Adesite SO, Tayo AO, Ashiru OA. (2008). Sesame radiatum phytoestrogenic lignans enhances testicular activity in Adult Male Sprague Dawley Rat Testis. Int. J. Morphology, 26 (3): 643- 652 .

WWF – UK News (2002). Oil spill threatens Spanish people and wild life.www.wwf.com accessed on the Nov. 15. 2002.

Zhang C, Qiao H (2004). Effect of polychlorinated biphenyls on spermatogenesis and testosterone secretion in adult cocks. J. Zhejiang Univ. Sci. 5(2): 193-197.