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Full Length Research Paper

Effect of Bonny light crude oil contaminated diet on serum cholesterol and testosterone concentrations in Wistar albino rats

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Crude oil pollution of the environment occurs on a daily basis through different modes. This has become even more pronounced in the oil-mineral producing areas of the Niger Delta of Nigeria. The purpose of this study was to investigate the possible effect of Bonny light crude oil on serum cholesterol and testosterone in Wistar albino rats, and to ascertain its possible endocrine disrupting effect on the reproductive hormone, testosterone. Eighty Wistar rats aged 15 to 17 weeks and weighing 193.6 to 220.0 g were used, and the experiment lasted for 28 days. The result showed that free testosterone was found to significantly decrease in all the groups among the male animals, except the control. However, a slight increase was observed in the female serum testosterone among the experimental groups. This increase was not significant ($p>0.05$). The result also showed that the cholesterol concentration in the males showed significant decrease ($p<0.05$), while in the females across the groups, significant increase in plasma cholesterol concentration were observed relative to the control group. We therefore suggest that Bonny light crude oil might have the potential to alter testosterone activity and may be a possible reproductive endocrine disruptor.

Key words: Testosterone, cholesterol, endocrine disruptors, fecundity, reproduction.

INTRODUCTION

Currently, widespread concern exists regarding the presence of pollutants in the environment which can interfere with normal endocrine functions in animals, including humans. Particular attention has focused on chemicals that are capable of mimicking or modulating the effects of gonadal hormones, thereby potentially interfering with the reproductive processes and behaviours of organisms (Doyle and Lim, 2002).

Traditional toxicological testing has focused on the ability of substances to cause obvious harm to adult or developing animals at high doses. Some endocrine disruptors are able to affect the hormonal system at very low doses, causing subtle disorders that may not be readily apparent or that may be delayed, even onto the next generation (IPCS, 2002). The discovery that chemicals could cause harm to animals and possibly to humans also in such subtle ways at low doses has challenged traditional views of toxicity and expanded gaps in toxicity testing in organism (Doyle and Lim, 2002).

The impact of crude oil spillage and discharge on the ecosystem as a result of oil exploration activities is an obvious problem of environmental concern (Otitoju and Onwurah, 2007; Ovuru et al., 2004). Similarly, the use of crude petroleum as therapeutic and in folkloric medicine in some parts of mineral oil producing communities in the Niger-Delta is another deliberate exposure with obvious health implications. Again, the exposure of workers in the oil industries may bring about a harvest of ill health, diseases and even death. There are accumulating evidences which indicate that humans, domestic and wildlife species are suffering from adverse health consequences from exposure to environmental chemicals that

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Table 1. The 4 sub-groups of the research plan.

Group	Treatment
Control	No crude oil in the feed
1.0%	Fed with 1.0 g of crude oil in 99.0 g of standard feed
5.0%	Fed with 5.0 g of crude oil in 95.0 g of standard feed
10.0%	Fed with 10.0 g of crude oil in 90.0 g of standard feed

interact with the endocrine system (Colbborn et al., 1993; Kavlock et al., 1996).

Cholesterol is a lipid synthesized by virtually all cells, especially the liver. It functions as structural component of membranes, precursors of bile salts, steroid hormones and vitamin D (Morrissey, 2006). Cholesterol in animal tissues is the most abundant member of a family of polycyclic compounds known as steroids. Although, many steroids are produced by the testes and the ovaries, the two most important are testosterone and estradiol. These compounds are under tight biosynthetic control, with short and long negative feedback loops that regulate the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary and gonadotropin releasing hormone (GnRH) by the hypothalamus (IPCS, 2002).

Defective synthesis of the steroid hormones produced by the adrenal cortex and the gonads can have profound effects on reproduction, human development and homeostasis. The aim of this study was to investigate the effect of crude oil contaminated-diet on serum testosterone concentration and cholesterol concentrations in Wistar rats.

MATERIALS AND METHODS

Test sample

The test sample for this study was Bonny light crude oil. The crude oil was obtained from the terminal of AGIP Okpoho Well 4, Nigerian National Petroleum Cooperation (NNPC).

Animals/experimental design

Eighty Wistar rats aged 15 to 17 weeks and weighing 193.6 to 220.0 g were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (UNN). They were acclimatized for one week at the Animal House of the Department of Biochemistry, Kogi State University, Anyigba. The rats were randomly divided into four groups; each group consists of ten rats (5 males and 5 females).

The research plan used consisted of 4 sub-groups labeled, control, 1.0, 5.0 and 10.0% as shown in Table 1.

Feed formulation

Different quantities of crude oil-contaminated diets (1.0, 5.0 and 10.0% w/w) were prepared by weighing out a definite amount of the crude oil and the feed and mixed in an HM 350S Binatone mixer at a speed of 100 rpm. About 5 ml of distilled water was added during mixing to facilitate homogenous mixing. The feed for the control group contained no crude oil.

Animal treatment/sample collection

Overnight, prior to exposure, the animals (rats) were starved of solid food and their body weight was taken. The rats were paired for mating and were also exposed to crude oil contaminated-diet from the first day of the experiment. All the animals were given standard rat chow and water *ad libitum* for 28 days. Then, they were anaesthetized and blood samples were collected for analysis. Blood (2 ml) was collected through the Ocular Median-Canthus vein of the rats with the aid of capillary tubes and transferred into test tubes containing EDTA (1.0 mM).

Gonadal hormone estimation

Concentrations of reproductive hormone, testosterone was determined in the serum using enzyme immunoassay kits (EIA), from Syntron Bioresearch Inc., USA. The experiment was carried out using the facilities of Arinkol Medical Laboratory Services, 46 Agbani Road, Enugu.

Serum samples (1.0 ml) were collected, frozen and stored in WestPoint deep freezer at -20°C for analysis.

Statistical analysis

Mean values (±SD) of replicate experiment (N = 2) were taken for each analysis. Significantly different results were established by one-way ANOVA (Duncan, 1955). The accepted level of significance is $p<0.05$.

RESULTS

The result showed that the mean concentration of cholesterol in the male group decreased significantly ($p<0.05$), while in the female groups, significant increase in plasma cholesterol concentration was observed relative to the control group (Figure 1).

Similarly, serum testosterone concentrations in both male and female Wistar rats showed significant decrease ($p<0.05$) in the mean value of serum testosterone in all the experimental groups of rats exposed to crude oil contaminated diet, especially the 1.0% (w/w) group (Figure 2). The highest value of testosterone (2.45 ± 0.83 ng/ml) was obtained in the males of the control group, while the females had 0.42 ± 0.18 ng/ml of testosterone. This highest value in the male rats is an indication of

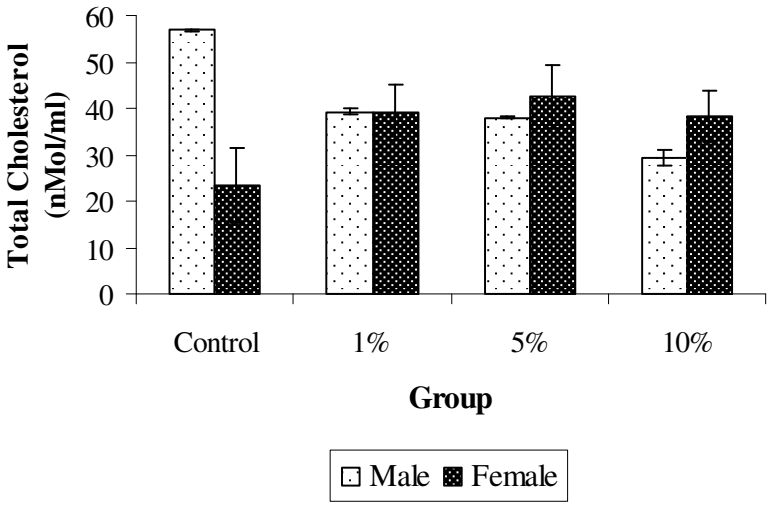

Figure 1. Mean serum cholesterol concentration of Wistar rats exposed to crude oil-contaminated diet.

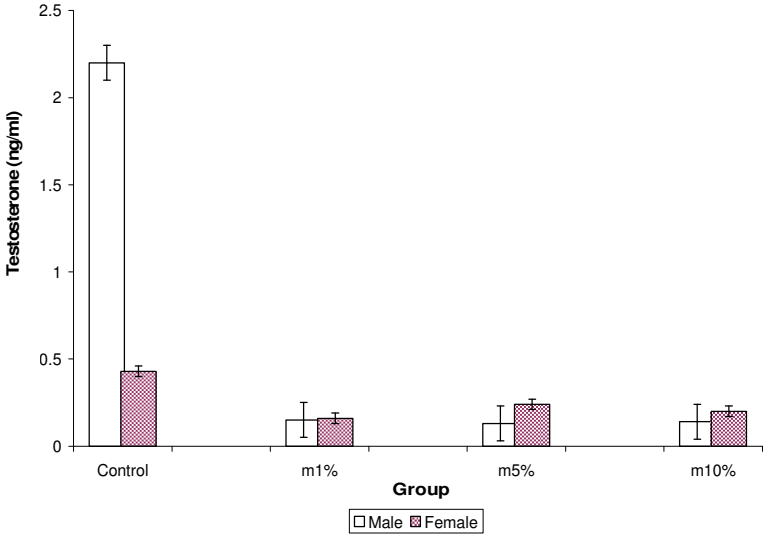

Figure 2. Serum testosterone concentrations of Wistar rats exposed to crude oil-contaminated diet.

gender specific hormone concentration. However, among the female species, serum testosterone appeared to significantly increase (p<0.05) among the 5.0 or 10.0% (w/w) groups. These results clearly show that some fractions of Bonny light crude oil could modulate the concentrations of free testosterone in the body of organisms that are exposed to its fraction(s) even at lower concentrations either in male or females organisms.

DISCUSSION

The results of this study highlight the potential susceptibility of Wistar rats to reproductive endocrine disruption following exposure to crude petroleum-contaminated diet, vis-a-vis serum cholesterol and serum testosterone concentrations. The role of cholesterol in steroidal hormones biosynthesis is a crucial point to note, as cholesterol is a major precursor in the synthesis of testosterone. The concentration of cholesterol was generally low in the experimental groups when compared to their control group. This reduced serum cholesterol concentration could elicit a lot of perturbation in the pathways responsible for the production of reproductive hormones. Polycyclic aromatic hydrocarbons (PAHs), the dominant constituents of crude oil, have also been implicated as EDs in fish, especially as modulators of

steroidogenesis (Evanson and Van der kraak, 2001). However, the major function of the liver in detoxification process is crucial to the concentration of cholesterol in the blood. Since the liver is also involved in the synthesis and metabolism of hormones, any alteration in the liver integrity may cause some anomalies in other metabolic processes that depend on the liver. For example, in humans and some other mammals, sex steroid testosterone circulate in the blood stream bound to sex hormone binding globulin (SHBG) and are thus, not freely available to target cells. Variation in the concentrations of SHBG (produced by the liver) will alter the biological activity of testosterone. Other factors that may affect the concentration of serum cholesterol may include sex of the animal/organism used. The male rats used in this experiment had reduced cholesterol concentration, while the female rats had increased cholesterol concentration when compared with their parallel control. This may imply that Bonny light crude oil or its constituent(s) may be eliciting its effects on cholesterol concentration based on differences in sex, which may affect some other processes, example metabolic rate and or growth rate.

The most commonly reported endocrine related effects of environmental contaminant exposure in wild life involve alterations in reproductive function and decreased reproductive success. Depending on the species and contaminant, these effects include development of male secondary sex characteristics in females, developmental abnormalities of the gonads, skewed sex ratios and same-sex pairing (Lorenzen et al., 2003). In this study, a decreased testosterone level in both male and female rats were observed, when compared with their control groups. Although, the testosterone concentration was higher in the male control group, the female experimental groups showed increased testosterone concentration when compared with their male counterparts.

Testosterone is primarily a male hormone but this steroid hormone is very important in females' health also. In males, low testosterone levels has been linked to decreased sex drive, impotence, infertility, abnormal breast enlargement, fatigue, depression and lowered irritability. Low testosterone level has been implicated in decreasing body hair, shrinking of testis and prostate (Chang et al., 2004). Of concern is, whether exposure to chemicals with steroid-like activity can disrupt normal endocrine function, leading to altered reproductive capacity, infertility, endometriosis and cancers of the breast, uterus and prostate (Jensen et al., 1995; Safe, 1995).

Vertebrates synthesize steroids via a pathway that involves the sequential degradation of cholesterol to progestins, then androgens (example testosterone) and finally oestrogens (example 17-oestradiol). This pathway is found in both sexes, and circulating plasma concentrations of sex steroids are representative of the relative conversion of androgens to oestrogens. Females have elevated plasma oestrogens when compared with androgens, whereas males have the opposite ratio. It is the ratio of androgens to oestrogens that creates a male versus female hormonal milieu (Guillette et al., 1994).

The endocrine system is a system of glands and the hormones they release regulates the development of a foetus in the womb, sexual development and reproductive function, maturation of the brain and nervous system, and energy metabolism (Ankley et al., 1997; Kavlock et al., 1996). Some researchers have postulated that a range of natural and synthetic chemicals in the environment could damage or disrupt human and animal endocrine systems at exposure levels much lower than what previous studies and regulatory agencies have determined to be dangerous or toxic. The result of this study revealed that the lowest concentration (1%) used, showed apparent disruption in the concentration of free testosterone in the exposed rats without showing any evidence of physical toxicity as compared with higher concentrations. This may be the reason why Schwartz (2002) adduced that regulatory agencies should set lower exposure limits for chemicals. This is intended to protect sensitive people from adverse effects due to chemical exposure, but proponents of additional regulatory safeguards believe that hormonally active chemicals could cause harm even at very low exposure levels. Hormones act at extremely low levels (part per trillion), therefore exposure to low levels of hormonally active agents as found in the crude oil may be of major health concern, particularly during sensitive periods of development and reproduction. Furthermore, endocrine-mediated effects may be subtle and manifest primarily in populations rather than in individuals.

This result shows that Bonny-light crude oil might have the potential to alter testosterone activity and may be a possible reproductive endocrine disruptor.

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