

Impact of Heavy Metals in Food Products from Crude Oil Polluted Area of Nigeria in Testicular Functions of Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author EOF designed and supervised the study. Author APE wrote the protocol, wrote the first draft of the manuscript, managed the literature searches, analyses of the study and performed the experimental process. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study aim at investigating the effect of crude oil contaminated food products on the reproductive function of male rats.

Study Design: Randomized controlled experiment.

Place and Duration of Study: Molecular Drug Metabolism and Toxicology Unit, Department of Biochemistry, University of Ibadan between January and April, 2013.

Methodology: Male rats were given food products from crude oil contaminated area (Imiringi-Bayelsa) and non crude oil contaminated area (Ibadan-Oyo) for 90 days. Commercial rat chow was given to the control group. Serum Follicle Stimulating Hormone (FSH), Leutenizing Hormone (LH), Testosterone, levels of heavy metals, Antioxidant status, Sperm parameters, and histology of testes were evaluated.

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Results: There was a decrease in Levels of FSH, LH and Testosterone by 58%, 66% and 82% respectively in testes and epididymis of rats exposed to crude oil contaminated feed when compared with control and non crude oil contaminated feed. Metal concentration in testes and epididymis showed the following arrangement: Pb>Ni>Cd, there was a concomitant increase in antioxidant enzymes, accompanied by increase in the levels of hydrogen peroxide and lipid peroxidation in Wistar rats that received food products from crude oil contaminated area, compared with control group and non crude oil contaminated area. Sperm parameters and histology of testes from all groups showed no observable difference.

Conclusion: Crude oil contaminated food products could induce oxidative stress in testes and epididymis of rats which may be as a result of metal accumulation, and this could alter normal spermatogenesis within the testicular micro environment at a longer period of exposure.

Keywords: Reproductive toxicity; testes; epididymis; metals; oxidative stress; rats.

1. INTRODUCTION

The components of crude oil include over 6000 potentially different hydrocarbon and metals [1,2]. These complexes have been confirmed to impact the soil at the occurrence of oil spillage which in turn accumulate in the biota of the environment, [3,4] and eventually find their way into the food chain.

Metal contaminants and environmental pollution have been reported to perturb the pro-oxidant/anti-oxidant arrangement of cells, thereby leading to generation of oxygen free radicals and reactive oxygen species (ROS) [5,6]. Elevation in the levels of ROS results in the oxidation of cellular components, with unsaturated fatty acid being the most vulnerable molecules. Mammalian spermatozoa, being rich in polyunsaturated fatty acids (PUFA), are more susceptible to oxidative stress, resulting in decrease in sperm motility and viability leading to infertility [7,8]. Induction of oxidative stress by components of crude oil such as Polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), heavy metals among others, have been a focal point of research in recent times [9,6,10].

Food products are often contaminated by numerous environmental chemicals through various processes. Many chemical contaminants (including dioxins, polyaromatic hydrocarbons (PAH), Polychlorinated biphenyls (PCBs), pesticides and some flame retardants), and heavy metals such as cadmium, found in contaminated soil tend to accumulate in fat tissue and milk fat [11]. Some chemical constituents of crude oil found in contaminated sites may also persist in the environment and build up in food chain [12].

Disorders of reproduction and hazards to reproductive health and associated functions become global concern in recent decades following reports of the adverse effects of certain chemicals on reproductive function. Recent scientific findings on human reproduction show tremendous increase cases of infertility among couples in industrialized countries [10] compared with couples during the early 1960s. Exposure of humans to disruptive chemicals appears to be related to various health problems, such as decreased fertility, but evidence is inconclusive [10]. Disorders of male reproductive function produce infertility, which may be permanent or temporary depending on the levels of exposure, duration of exposure, and nature of the toxicants [13].

Considering that humans can be exposed to crude oil via multiple sources, it is crucial to evaluate the effect of the secondary exposure of this compound in animal model. Our previous studies revealed that, direct exposure to crude oil can disrupt antioxidant status in serum, brain, liver, kidney, testes and sperm, accompanied by decrease sperm count, altered sperm motility and morphology and increase abnormality in sperm cells, induced endocrine disruption and alteration in testicular stress response proteins and caspase-3 dependent apoptosis in albino Wistar rats [10,13-17]. Moreover previous studies elsewhere showed that rats exposed to shell fish from crude oil contaminated site for twenty-eight days resulted in haematotoxicity [18].

To the best of our knowledge, this study is the first to investigate the effects of crude oil contamination of food products on hormonal profile, histology and antioxidant status in testes and epididymis of rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Nicotinamide adenine dinucleotide phosphate (NADP); epinephrine; glutathione (GSH); 5, 5-dithio-bis-2-nitrobenzoic acid; hydrogen peroxide; thiobarbituric acid; and 1-chloro-2, 4-dinitrobenzoic acids were purchased from Sigma Chemical CO (USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (UK).

2.2 Animal Protocol

Thirty-six healthy male Wistar rats weighing approximately 30 g \pm 2 g, were obtained from the Primate colony of the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. They were randomly assigned to 3 groups of 12 animals each. Animals were housed in suspended plastic cages placed in a well-ventilated rat house and were provided with feed from natural food products obtained from crude oil polluted area and non crude oil polluted area of Nigeria for 90 days, and water ad libitum, and subjected to a 12:12 light-to-dark photoperiod. African cat fish (*Clarias gariepinus*); Maize (*Zea mize*); and fluted pumpkin (*Telfairia occidentalis*), from crude oil polluted swamp (Kolo creek, Imiringi) were used to formulate feed given to animals in test group (COP). However; a corresponding group of fish (*Clarias gariepinus*) obtained from a clean pond from ZATECH group of company in Ibadan; maize and fluted pumpkin (*Telfairia occidentalis*) harvested from farms in Ibadan (non crude oil contaminated farm) were given to the animals in non crude oil contaminated group (NCOP). Other food supplements were obtained from vet needs, a subsidiary of ZATECH group of company in Ibadan, while the commercial rat chow from ZATECH group of company Ibadan, were given to control group.

The food composition include 20% fish meal (*Clarias gariepinus*), 33% cornstarch (*Zea mize*), 33% whole grain (*Zea mize*), 07% fluted pumpkin (*Telfairia occidentalis*), 06% vegetable oil, 0.4% NaCl, 0.6% mixture of minerals and vitamins. Food products for this study were obtained from Ibadan, non crude oil polluted area, (Latitude: 7° 24' 45" N Longitude: 3° 55' 45" E, Western Nigeria), and Imiringi (Latitude: 4° 8' 53"N Longitude: 6° 3' 71"E Southern Nigeria). Food products were prepared in pellet form

within 2 weeks interval throughout the 90 days of feeding.

2.3 Measurement of Water Intake

Daily water intake was obtained using a measuring cylinder and a calibrated conical flask. The daily water intake was obtained by subtracting the volume of water remaining at the end of 24 hours of feeding from the initial amount in the cylinder at the start of each day's feeding. There was no variation in water intake across group.

2.4 Measurement of Food Intake

Daily food intake was obtained by giving a measured quantity of rat feed; each day and weighing the quantity remaining, same time the next day. The difference in quantity was recorded as the food intake. The recording was done at the same time daily to ensure consistency and accuracy. Each group received between 15-20g of feed per day. Weights of the animals were observed at the end of each week during the period. There was no observable difference in body weight and food intake across group.

All animals received humane care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institutes of Health [19], as ethical regulations were followed in accordance with National and Institutional Guidelines for the Protection of Animal Welfare during the experiments. At the end of 90 days feeding, the weight of rats were taken and sacrificed by cervical dislocation; testes and epididymis were quickly removed, weighed and stored at -20°C until analysis.

2.5 Biochemical Assay

Twelve testes and epididymis from control, non crude oil contaminated group and crude oil contaminated group were separately homogenized in 50 mM of Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate was centrifuged at 10,000 x g for 15 min at 4°C. The supernatant was collected for the estimation of catalase (CAT) activity using hydrogen peroxide as substrate according to the method of Clairborne [20]. Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich [21]. Glutathione peroxidase

(GSH-Px) was assayed by the method of Paglia and Valentine [22]. Protein concentrations were determined by the method of Lowry et al. [23]. Reduced GSH was determined at 412 nm using the method described by Jollow et al. [24]. Hydrogen peroxide generation was assessed by the method of Wolff [25]. Lipid peroxidation was quantified as MDA according to the method described by Farombi et al. [26] and expressed as μmol MDA formed/g tissue.

2.6 Hormone Analysis

Blood samples taken for hormone analysis were centrifuged at $3500 \times g$ for 15 min and serum was extracted. Serum FSH, LH and Testosterone levels were measured by the enzyme-linked immunosorbent assay (ELISA).

2.7 Histology

Samples from testes were fixed with Bouin's solution, sectioned, and stained routinely with haematoxylin and eosin for microscopy. All slides were coded before examination using a light microscope.

2.8 Sperm Motility Assay

Sperm motility was assessed by the method described by Zemjanis [27]. The motility of epididymal sperm was evaluated microscopically within 2–4 min of their isolation from the cauda epididymis, and data were expressed as percentages according to Pant and Srivastava [28].

2.9 Determination of Daily Sperm Production and Testicular Sperm Number

Daily sperm production was determined using twelve frozen left testes from control and treated rats according to Joyce et al. [29]. Briefly, after the testes were removed and weighed, they were homogenized for 3 min in 25 ml physiologic saline containing 0.05% (v/v) Triton X-100. Sample aliquots of the $5.5 \mu\text{l}$ were then placed on the haemocytometer and counted twice at 100 magnifications under a microscope to determine the average number of spermatids per sample. These values were used to obtain the total number of spermatids per testis, and this number was then divided by the testes weight to yield spermatids per gram of testes. Developing spermatids spend 4.61 days in rats. Therefore,

the values for the number of spermatids per testis were divided by 4.61 to obtain daily sperm production.

2.10 Morphologic Abnormalities and Percentage Viability Assay

A portion of the sperm suspension placed on a glass slide was smeared out with another slide and (1) stained with Wells and Awa's stain (0.2 g eosin and 0.6 g fast green dissolved in distilled water and ethanol in a 2:1 ratio) for morphologic examination and (2) stained with 1% eosin and 5% nigrosine in 3% sodium citrate dehydrate solution for live-to-dead ratio according to the method described by Wells and Awa [30].

2.11 Determination of Heavy Metals in Tissues and Feeds

The levels of, Pb, Cd and Ni in the post mitochondria fraction of the tissues (testes and epididymis) and feeds were determined with the aid of Atomic Absorption Spectrophotometer A Analyzer 200 (USA) according to AOAC [31]. An acetylene air mixture was used as the flame. The working standard for each of the metals were aspirated into the flame in the order of 0.0 ppm, 0.8 ppm and 1.6 ppm. The samples were then aspirated into the flame and the values were obtained.

2.12 Statistical Analysis

Statistical analysis were carried out using one-way analysis of variance, followed by Tukey's post-test using SPSS (student version 17.0, SPSS Inc., UK) to compare experimental groups; $p < 0.05$ was considered statistically significant.

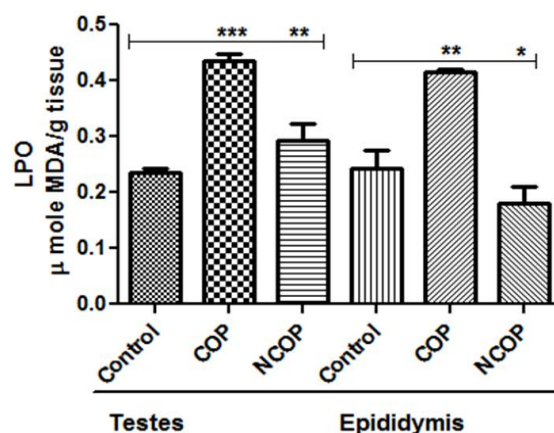
3. RESULTS

3.1 Biochemical Studies

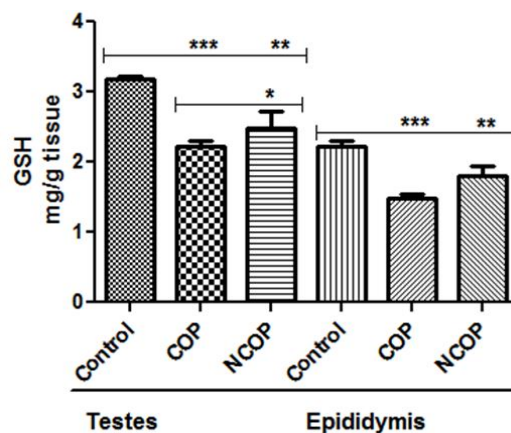
Rats exposed to food products from crude oil contaminated area exerted a significant ($p < 0.05$) increase in testes and epididymal lipid peroxidation (LPO) by 40% and 70% respectively relative to control and non crude oil contaminated area. There was decrease in GSH levels when compared with rats that received commercial rat chow and group that received food products from non crude oil polluted area by 55% and 120% respectively (Fig. 1A and B). The activities of

SOD and CAT in testes and epididymis increased significantly ($p < 0.05$) in group given crude oil contaminated food samples when compared with group exposed to commercial rat chow and feed from non crude oil polluted area by 60% and 80% for SOD and by 30% and 35% for CAT respectively (Fig. 2A and B). There was a significant increase in testes and epididymal H_2O_2 generation in rats exposed to crude oil

contaminated food products relative to control and non crude oil contaminated site by 35% and 40% respectively (Fig. 3B). Furthermore there was a significant ($p < 0.05$) increase in the GSH-Px activities in testes and epididymis of rats exposed to crude oil contaminated feed when compared with the group from control and non crude oil polluted area by 25% and 30% respectively (Fig. 3A).



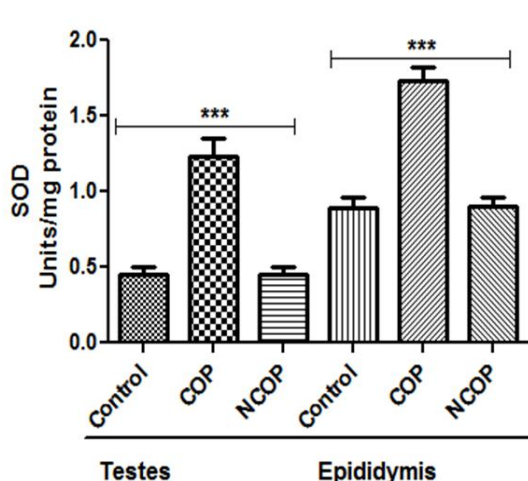
(1A)



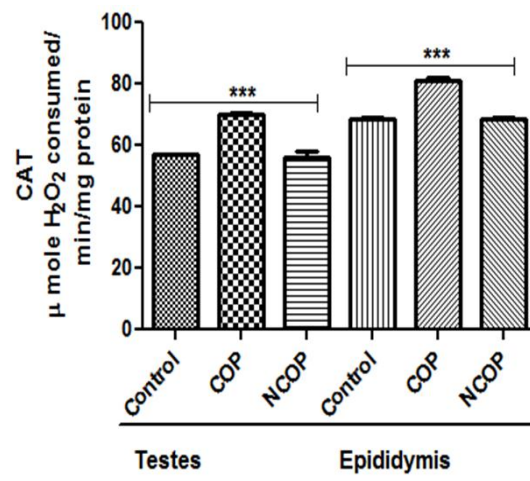
(1B)

Fig. 1A and B. Effects of crude oil contaminated feed on testes and epididymal LPO and GSH levels in rats.

The data are expressed as mean \pm SEM; $n=12$. * values differ significantly from control ($p < 0.05$). Control (commercial rat chow), COP (feed from Bayelsa {crude oil contaminated area}) and NCOP (feed from Ibadan {non crude oil contaminated area}).



(2A)



(2B)

Fig. 2A and B. Effects of crude oil contaminated feed on testes and epididymal SOD and CAT activities in rats.

The data are expressed as mean \pm SEM; $n=12$. * values differ significantly from control ($p < 0.005$). Control (commercial rat chow), COP (feed from Bayelsa {crude oil polluted area}) and NCOP (feed from Ibadan {non crude oil contaminated area}).

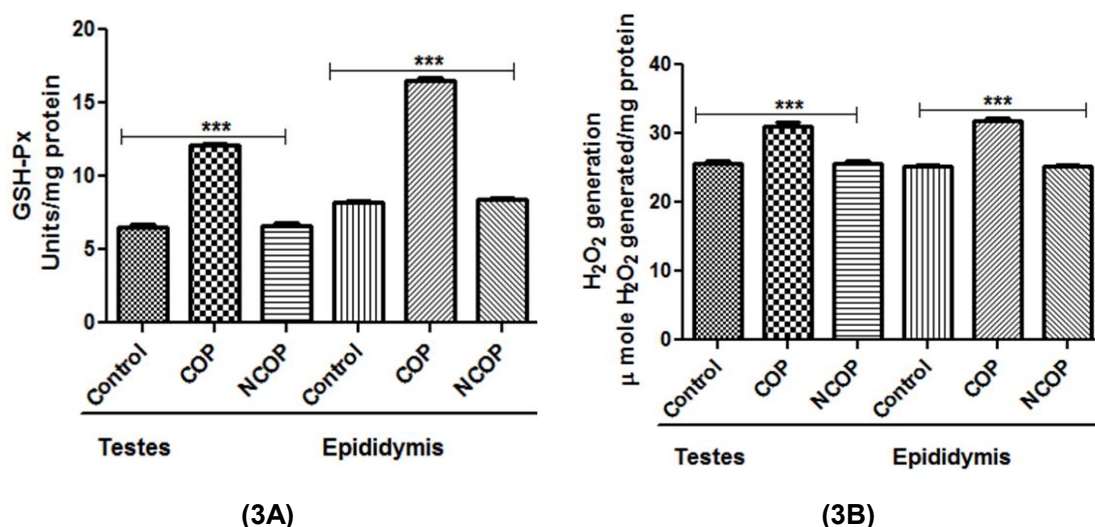


Fig. 3A and B. Effects of crude oil contaminated feed on testes and epididymal GSH-Px activity and H₂O₂ level in rats

The data are expressed as mean±SEM; n=12. *values differ significantly from control (p<0.05). Control (commercial rat chow), COP (feed from Bayelsa {crude oil contaminated area}) and NCOP (feed from Ibadan {non crude oil contaminated area})

3.2 Hormone Analysis

After 90 days feeding we observed a marked decrease in serum FSH, LH and testosterone levels in group that received food products from contaminated site relative to control and non contaminated site (Table 1).

Table 1. Hormone level (ng /ml)

	FSH	LH	Testosterone
Control	2.01±0.11	1.96±0.02	1.34±0.13
COP	0.79±0.01*	0.97±0.03*	0.20±0.03*
NCOP	1.96±0.25	1.63±0.06	1.10±0.35

n = 12, mean ± SEM, *: P < 0.05 Vs the control group.
Control (commercial rat chow), COP (feed from Bayelsa {crude oil polluted area}) and NCOP (feed from Ibadan {non crude oil polluted area})

3.3 Histology

Good capsule architecture and well-arranged seminiferous tubules were observed in testes of rats among groups during the experiment (Fig. 4A, B and C).

3.4 Sperm Parameters

The result of sperm count, motility, live/dead ratio, daily sperm production and total abnormality revealed no observable difference across the three groups (Fig. 5).

3.5 Metal Concentrations in Testes, Epididymis and Feeds

Fig. 6 showed the levels of metals in the testes and epididymis. There was marked accumulation of Pb, Cd and Ni in testes and epididymis of rats exposed to food products from crude oil contaminated site when compared with control and non contaminated site. Table 2 showed higher heavy metal concentration on compounded feed from crude oil contaminated site when compared with commercial feed and non crude oil contaminated feed.

4. DISCUSSION

People residing in areas that are continually experiencing environmental pollution from industries and hazardous waste sites are, highly exposed to chemicals such as benzo (a) pyrene, atrazine, PAHs, PCBs, heavy metals among others [6,32,33]. Impact of oil spills need to be viewed in the context of stress on the many marine and coastal ecosystems, communities and populations that are already chronically depleted or weakened by industrial pollution [34]. Several communities in the Niger delta area of Nigeria have experienced oil spill in recent times; among the prominent site of pollution is Immiringi-Bayelsa which is our area of study.

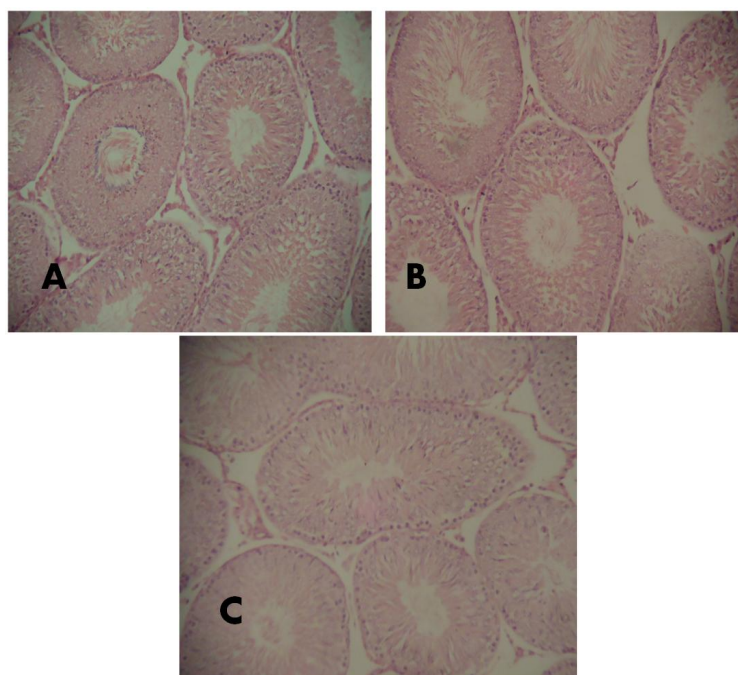


Fig. 4A, B and C. The testis section in control A; showing no visible lision, B (COP); no visible lision and C (NCOP); no visible lision
H&E X100, Showing no damage to the normal architecture in all groups. Control (commercial rat chaw), COP (feed from Bayelsa {crude oil polluted area}) and NCOP (feed from Ibadan {non crude oil polluted area})

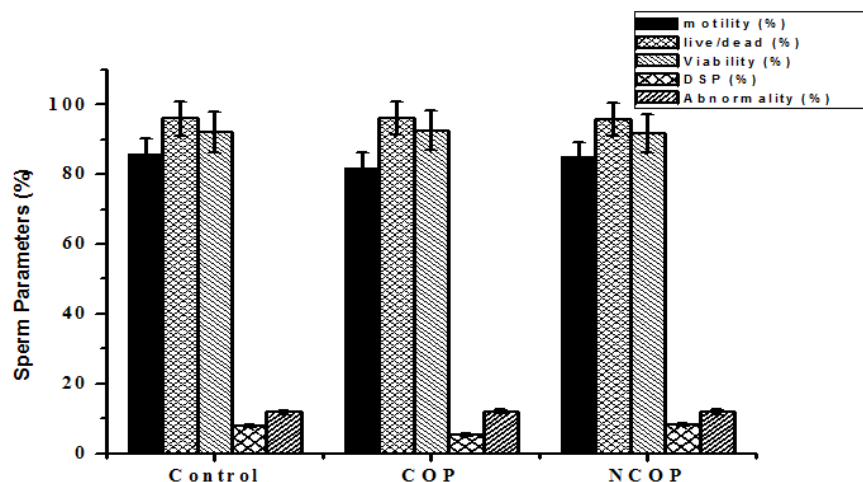


Fig. 5. Crude oil contaminated feed showing no observable change in sperm parameters when compared with non crude oil contaminated feed and control

*The data are expressed as mean±SEM; n=12. * values differ significantly from control (p<0.05). Control (commercial rat chaw), COP (feed from Bayelsa {crude oil polluted area}) and NCOP (feed from Ibadan {non crude oil polluted area})*

The testicular system is susceptible to many chemicals/pollutants including pesticides, PAHs, PCBs, heavy metals and other environmental contaminants associated with crude oil pollution

[35]. Accumulation of metals in food products and plants (vegetables), of crude oil contaminated area is well known [36,37] and this corroborates the result from current study.

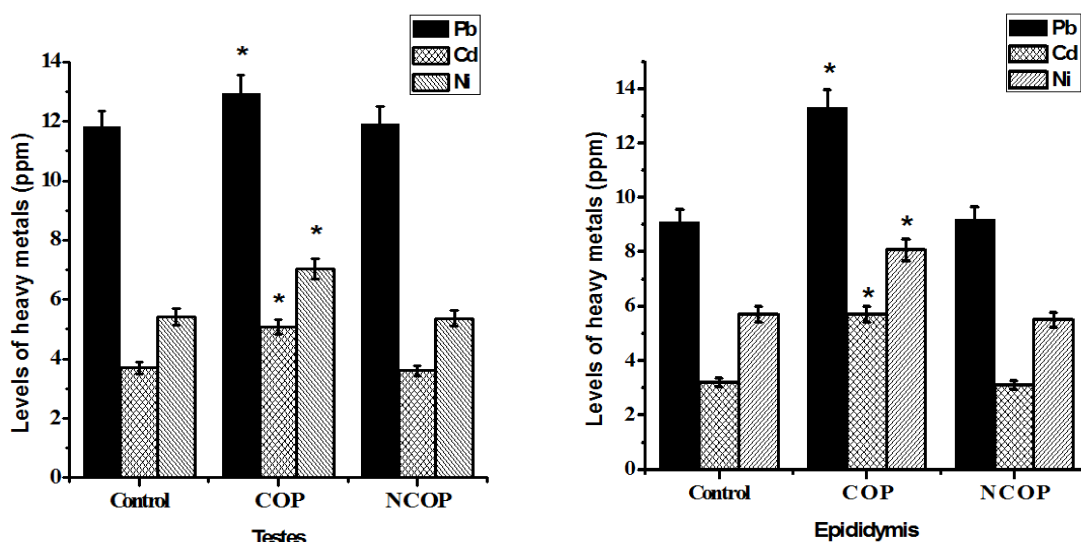


Fig. 6. Crude oil contaminated feed (COP) showing significant increase in concentration of metals in testes and epididymis when compared with control and non crude oil contaminated feed (NCOP)

The data are expressed as mean \pm SEM; n=12. * values differ significantly from control ($p < 0.005$). Control (commercial rat chow), COP (feed from Bayelsa {crude oil polluted area}) and NCOP (feed from Ibadan {non crude oil contaminated area})

Table 2. Metal concentration in compounded feed and commercial feed

Metals	Control	COP	NCOP
Pb (ppm)	0.03 \pm 0.0001	0.70 \pm 0.0002*	0.04 \pm 0.0001
Cd (ppm)	0.01 \pm 0.0001	0.30 \pm 0.0001*	0.012 \pm 0.0001
Ni (ppm)	0.012 \pm 0.001	0.24 \pm 0.0001*	0.013 \pm 0.0001

n = 12, mean \pm SEM., *: $P < 0.05$ Vs the control group. Control; commercial feed; COP; Compounded feed from Bayelsa (crude oil polluted area); and NCOP; Compounded feed from Ibadan (non-crude oil polluted area)

The fundamental role of oxidative stress in the onset of defective sperm function via mechanisms involving the induction of peroxidative damage to the plasma membrane has been reported [38,10]. Levels of LPO and H_2O_2 were elevated significantly in testis and epididymis of rats exposed to food products from crude oil contaminated area, compared with control and non crude oil contaminated site. The significant increase in LPO and H_2O_2 observed in this study may be a response to oxidative stress caused by the presence of heavy metal accumulation observed in the food products from crude oil contaminated area which is in agreement with previous studies elsewhere [36,37]. Accumulation of metals have been shown to induce superoxide dismutase (SOD) which converts superoxide radical to H_2O_2 and concomitantly increase catalase (CAT) activity that acts in tandem with superoxide dismutase [10,14], this is in agreement with the current study. The decreased levels of antioxidant GSH

could be the reason for the significant increase in GSH-Px activity which utilizes GSH as a substrate in combating the fluctuation of superoxide radicals in the system and/or may indicate a stress response of the tissues to metals/environmental pollutants [10, 14]. Increase in the activity of CAT and SOD is usually observed in response to the toxicity of environmental pollutants [39,40]. Oxidative damage to macromolecules as a mechanism of Cd toxicity has been reported [41]. Cadmium has also been shown to have anti steroidogenic effects [14] which support the decrease level of testosterone observed in this study. Current study showed that there was an accumulation of Cd, Pb and Ni, in the testes and epididymis of rats exposed to food products from crude oil impacted area relative to control and group from non crude oil contaminated site. This is in agreement with our previous report on direct exposure to crude oil [14] "Tissues distribution of heavy metals and erythrocytes antioxidant status

in rats exposed to Nigerian bonny light crude oil". Heavy metals are of extensive environmental concern due to their toxicity and cumulative behavior [42]. Incidence of metallic poisoning arising from contamination of water, soil, and food products are well documented [43]. This obvious oxidant/antioxidant imbalance in the testis and epididymis of rats exposed to food products from crude oil impacted area in this study may be attributed to the accumulation of metals in the food products and other components of crude oil that have the ability to persist in the environment after oil spill.

Reports showed that androgen deprivation induces lipid peroxidation in rat testes [44]. Testosterone, secreted almost exclusively by Leydig cells in the testis, is the primary steroid hormone that maintains male fertility. Leydig cell testosterone biosynthesis is regulated by the pituitary gonadotropin LH [45,8]. The decreased serum concentrations of FSH and LH in this study suggest adverse effect of crude oil contaminated food products on hormone homeostasis. Decrease in serum testosterone level in the present investigation is a reflection of the adverse effect of 90 days exposure to food products from crude oil contaminated site, on steroidogenesis and has strengthened the phenomenon of generation of oxidative stress in testicular and epididymal tissues. LH is required for initiation and maintenance of quantitatively normal spermatogenesis in pubertal rats [46]. Testosterone is needed for the maintenance of the spermatogenic process and inhibition of germ cell apoptosis [8]. The effects on testosterone may be attributed to the decrease in serum LH, since LH serves as a normal stimulus for the secretion of this steroid from the testicular Leydig cells [14].

Overall, there was no observable change in sperm parameters after 90 days exposure to food products from crude oil contaminated area. Moreover histology observations showed no damage in the testes of rats exposed to food products from crude oil contaminated area. Interestingly it has been reported that obvious toxicology risk can occur without physical damage to tissues, for the reason of adaptive mechanism [33].

5. CONCLUSION

This data provide valuable information on toxicology risk associated with metals and other

components of crude oil contamination on food products, in the testes and epididymis of rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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