Effects of Ingestion of Crude Oil-Contaminated Diets on Organs' Weights and Histopathology of the Pig

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Abstract

The effects of ingestion of crude oil-contaminated diets were investigated on weights of selected organs, namely: liver, heart, kidney and spleen of the growing pig. Forty-eight landrace weaner pigs weighing on average 10 ± 1.1kg were used. The pigs on arrival at the animal wing of the Teaching and Research Farm of the Rivers State University were randomly assigned to four dietary treatment groups of 12 pigs/group. The animals were properly de-wormed via ivermectin injection subcutaneously and a broad-spectrum antibiotic (amoxicillin) intramuscularly. At the end of pre-conditioning, animals were served with their experimental diets: treatment 1 (T_1, control diet, had 0 gram of crude/kg of diet, treatment 2 (T_2, 5 gram/kg), treatment 3 (T_3,10 gram/kg) and treatment 4 (T_4, 15 gram /kg). Experimental diets were served at 5% of individual animal body weights for 4 weeks, based on a completely randomized design (CRD). At the end of the 4-weeks' study period, 4 pigs from each dietary treatment group were humanely slaughtered and organs of interests were removed and weighed, then were dissected for histopathology assessments. Ingestion of crude oil-contaminated diets had no effects ($P > 0.05$) on hearts and spleens of the animals but significantly ($P < 0.05$) affected the livers and kidneys resulting in neoplasia in these organs, especially for treatments 3 and 4 as confirmed by the histopathological examinations of the organs. It was concluded that ingestion of crude oil-

contaminated diets affected the livers and kidneys of pigs, especially at dietary treatments 3 and 4 but not the hearts and spleens.

Key words: Crude oil, Contamination, Organ weights, Histopathology and Pig.

Introduction

Crude oil is a complex mixture of hydrocarbons from which various petroleum products, such as gasoline, kerosene, lubricating oil, wax and asphalt are derived (Igwe and Lee, 2014). Crude oil has been reported to be a potent toxicant as a result of its toxic components such as aliphatic, alicyclic and polycyclic aromatic hydrocarbons and its water soluble fractions that have been implicated in some of the adverse impacts of crude oil in animals and humans (Egborge, 1991). To this point, therefore, the choice of the animal model for this study is the pig. The pig like humans is an omnivorous animal and most of its major structures anatomically and physiologically are very similar with those of humans, indicating that results obtained with the pig can be used to explain at least in part similar conditions in humans. For instances, anatomically and physiologically, pigs are very similar to humans in the cardiovascular and pulmonary systems. Furthermore, pigs are non-ruminant omnivores and exhibit similarities to humans in the digestive physiology, nutrient requirements and susceptibility to a host of gastro-intestinal pathogens and toxicants (Alam *et al.* 2010). Thus, science has come to an agreement that if 'something' works in the pig, it would also work in humans thereby making the pig model as the choice animal model for biomedical research such as this study; leading to the pig being regarded as the 'best translational animal biomedical research model' (NRC,2012).

To our knowledge, no study has investigated the effects of ingested crude oil-contaminated diets on organ weights and histopathology on liver, kidney, heart and spleen of the pig. Therefore, the objectives of this study are to examine the effects of ingested crude oil-contaminated diets on the weights the liver, kidney, heart and spleen of the pig as well as the effects of the ingested crude oil-contaminated diets on the histopathology of these organs.

Materials and Methods

Experimental Animals and Management: 48 landrace weaner pigs weighing on the average 10 ± 1.1kg were acquired from Cape Farms, Irete, Imo State, Nigeria. The animals on arrival at the Teaching and Research Farm of the Rivers State University, were randomly assigned to four treatment groups of 12 pigs per treatment group. The animals were then allowed to be fully adapted to their new environment for 14 days to stabilize. During this period, all animals in the groups were similarly managed. They were fed similar diet and water was provided *ad libitum*. Also, during this period of pre-conditioning, the animals were properly de-wormed using ivermectin injection subcutaneously. They were also given a broad-spectrum antibiotic (amoxicillin) intramuscularly. These protocols were adopted to ensure sound health status of the animals before commencing the study proper being a biomedical study.

Crude Oil Contamination, Experimental Diets and Design: The crude oil used in this study was Bonny Light obtained from Agip Oil Company Nigeria Ltd. Omoku, Rivers State. Before the crude oil was incorporated into the diets, it was exposed to sunlight in shallow pans for 24 hr. to enable evaporation of the light and volatile fractions to simulate the natural condition during oil spillage according to the method of Berepubo *et al*. (1994).

The feed used in this study was Pfizer pig grower mash^{TM}. Crude oil was added at 4 different concentrations to the grower mash as: treatment 1 (T_1, contained 0 gram of crude/kg of diet) and therefore served as the negative control diet, treatment 2 (T_2, contained 5 gram of crude oil/kg of diet), treatment 3 (T_3, contained 10 gram of crude oil/kg of diet) and treatment 4 (T_4, contained 15 gram of crude oil/kg of diet), respectively. Experimental diets were served at 5% of individual animal weights for 4 weeks. The design used was a CRD of 12 replications per dietary treatment.

Organ Collection for Weights Determination: At the end of the study period, 4 pigs from each dietary treatment group were randomly selected and humanely slaughtered by restraining. Bleeding was done by severing the carotid vein with a sharp knife and blood was allowed to be completely drained. The animals were then eviscerated and the organs of interest, namely: heart, liver, kidney and spleen were harvested and weighed and their weights were recorded. The weights of the organs were determined using Camry Electronic Kitchen Scale (Mo del Ek 3052).

Organ Histopathological Examination: The already weighed organs were later dissected according to the method of Suri *et al*. (2014) at the University of Port Harcourt Teaching Hospital. Briefly, organs harvested were placed into storage vials containing 10% buffered formaldehyde solution to maintain organ integrity before sectioning. Prepared slides were filmed using a photomicrograph camera to produce plates of the organs at 200 and 400 magnifications.

Data Analysis: Data obtained were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure of SAS. Organ weights were compared using Tukey's test. The model was: $Y_{ij} = \mu + X_i + E_{ij}$, where Y_{ij}= individual observation of the treatment, μ = population mean, X_i = effect of the i^{th} treatment and E_{ij} = the error term. An *α-level* of *0.05* was used for all statistical comparisons to represent significance.

Results

The results of the relative organ weights of pigs fed varying levels of crude oil-contaminated diets are shown in **Table 1**.

Table 1. Effects of different levels of crude oil-contaminated diets on relative organ weights of pigs

	TREATMENTS					
Organs	**T_1**	**T_2**	**T_3**	**T_4**	SEM	*P-value*
Liver (g)	2.82^a	3.20^a	3.40^a	3.85^b	0.13	0.007
Heart (g)	0.50	0.49	0.47	0.48	0.10	0.267
Kidney (g)	0.47^a	0.51^a	0.58^b	0.59^b	0.02	0.005
Spleen (g)	0.20	0.16	0.20	0.21	0.02	0.083

^{a,b}means within the same row with different superscripts are significantly different ($P < 0.05$)

Ingestion of crude oil-contaminated diets had significant ($P < 0.05$) effects on the livers' and kidneys' weights of the animals that consumed the different crude oil-contaminated diets but not on the heart and spleen as there were no significant ($P > 0.05$) differences amongst dietary treatments. Accordingly, therefore, animals that ingested diets 1, 2 and 3 had similar liver weights which were significantly ($P < 0.05$) lower compared to the livers of animals on diet 4, that had the highest crude oil level in their diet. Again, while animals on diets 1 and 2 had similar

($P > 0.05$) kidney weights, animals on diets 3 and 4 had significantly ($P< 0.05$) higher kidney weights compared with kidneys of pigs with lower concentrations of dietary crude oil intake.

The histopathological slides of the hearts of animals in all treatment groups showed normal cardiac muscle and blood vessels. Similarly, histologic section from the spleen showed normal spleen histology for all dietary treatment groups. However, for the livers, while livers of animals on diets 1 and 2 demonstrated normalcy for the fact that no obvious histologic changes were observed in the central vein, portal tract and hepatocytes; for livers of animals on dietary treatments 3 and 4, mild vacuolar changes, sinusoidal dilation and inflammatory cells were observed, but in addition for livers on treatment 4, their livers demonstrated major vacuolar changes and congested vessels.

Discussion

Organ weights analyses are very important in any toxicity studies involving animals. It has been demonstrated that prolonged ingestion of crude oil or its refined counterparts, crude oil polluted plants, seeds and water by animals affects animal cells, tissues and organs by which crude oil influences the growth and performance of the animal, especially when the functionality of a given organ increases leading to atrophy of the organ and by vice versa. To this extent, Heywood (1981) reported reduction in organ weights amongst groups of rats exposed to crude oil in his toxicological studies. He further highlighted that organs in respect to their weights responded to different chemicals contained in crude oil by 84% in their rodent studies and 65% in dog studies. Furthermore, the data of Heywood (1981) also demonstrated that the liver was affected by 56% of the chemicals found in crude oil. He therefore concluded that increase or decrease in organ weights could be an adaptive response or pathologically induced by the ingested toxicant.

Moles and Rice (1983) posited that chemical products, such as those of crude oil are environmental stressors that cause body organ atrophy or hypertrophy. This was observed in this current study in livers and kidneys of the pigs that ingested dietary crude oil-contaminated diets, particularly for treatments 3 and 4 showed neoplasia in a linear fashion as the levels of dietary crude oil increased. These data were further substantiated to in the studies of Ngodigha *et al*. (1999) that demonstrated linear decrease in the weights of the heart due to the toxic effect of

crude oil but as crude oil contamination in the feed increased there were linear increases in the weights of liver of West African Dwarf Goats. The authors therefore concluded that increased level of crude oil ingestion by the goats caused liver hyperplasia. These findings of Ngodigha *et al*. (1999) were in tandem with our findings in this study. The data of Akporhuaho, (2011) in poultry further support our current findings of organ hyperplasia. Berepubo *et al*. (1994) that investigated the effect of feeding crude oil contaminated feeds to weaner rabbits reported significant reductions in the size/weights of the liver, kidney, heart, pancreas and testes of the rabbits in a dose dependent manner. Similarly, Nodu and Ohimain (2014) reported atrophy of the liver and kidney of rabbits exposed to crude oil contaminated forage. These observations were also confirmed in our study, indicating agreement with their data.

Histopathological investigation of organs has been a well-known technology used in affirming morphological changes especially in toxicological studies to properly deduce the effect of the toxicant, such as crude oil on the organs studied. Our findings in this study with particular emphases on the liver of the pigs on the higher levels (treatments 3 and 4) showed some forms of structural disintegration in the hepatocytes from the micrographs supporting the fact that crude oil consumption portends serious damaging effects on the liver. Similar observations were also found in the studies of Sumonu and Oloyede (2007) and George and Sese (2012). These adverse effects of crude oil ingestion on animal organs, such as the liver might be used to explain at least in part poor growth performances in animals involving crude oil ingestion.

Conclusions

The ingestion of crude oil-contaminated diets, especially at higher levels of ingestion in this study resulted in organ atrophies in the liver and kidneys of growing pigs as confirmed by morphological changes in these organs.

References

Akporhuaho, P. O. (2011). Effects of crude oil polluted water on the haematology of cockerel reared under semi-intensive system. *International Journal of Poultry Science 10 (7): 527-529.*

Alam, J. B. Ahmed, A. A. and Munna, G. M. (2010). Environmental impact assessment of oil and gas sector: A Case study of Magurchara Gas Field. *Journal of Soil Science and Environmental Management. 1(5): 86-92.*

Berepubo, N. A. Johnson, N. C. and Sese, B. T. (1994). Growth potentials and organ weights of weaner rabbits exposed to crude oil contaminated forage. *International Journal Anim. Science. 9: 73-76.*

Egborge, A. B. (1991). Industrialization and heavy metal pollution in Warri River. *32nd Inaugural Lecture University of Benin, Benin City, Nigeria.*

George, O. S. and Sese, B. T. (2012). Histopathological changes in the liver and kidney of adult buck rabbits exposed to crude oil contaminated feed. *Global Journal Biological-Science Biotechnology 1(2):277-280.*

Heywood, R. (1981). Target organ toxicity. *x* 8:349-358.

Igwe, F. U. and Lee, G. D. (2014). Dermal toxicity studies of adult albino rats exposed to Bonny Light crude oil. *Journal of Nigerian Environmental Society. 1 (1):27-28.*

Moles, A. and Rice, S. D. (1983). Effects of crude oil and naphthalene on growth, caloric and fat contents of pink salmon juvenile in sea water. *Trans American Fish Society. 112(2): 205-211.*

Ngodigha, E. M. Olayinka, F. O. Oruwari, B. M. Ekweozor, I. K. E. and Wekhe, S. N. (1999). Toxic effects of crude oil on organ weights and blood cells of West African Dwarf Goat. *Nigerian Veterinary Journal. 20 (1):82-91.*

Nodu, M. B. and Ohimain, E. (2014). The effects of crude oil contaminated forage on breeding behavior and reproductive performance of female rabbits. *International Journal of Plant, Animal Environmental. Science 4:2-7.*

NRC (2012). Nutrient Requirements of Swine. 11th Edition, National Academic press Washington, D.C.

Sumonu, T. O. and Oloyede, O. B. (2007). Biochemical Assessment of the effects of crude oil contaminated feed on the hepatocytes and performance of cat fish (*Clarias gariepinus*). *Afriican Journal of Biochemistry. 1(5):083-089.*

Suri, K. Iyer, R. Patel, U. Bandil, S. (2014). Histopathology and Clinico-histopathological correlation in Hansen's disease. *Journal of Research Medical Dental Science. (1): 37-44.*