©IDOSR PUBLICATIONS
International Digital Organization for Scientific Research ISSN: 2579-0781
IDOSR JOURNAL OF EXPERIMENTAL SCIENCES 5(1) 53-59, 2020.

Effects of Water Soluble Fractions (WSF) of Crude Oil on Reproductive Performances of Pregnant Wistar Rats (*Rattus norvegicus*)

Okoroigbo F. I., Zitte L. F. and Agu G.O.

Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria.
Email: Leelee.zitte@uniport.edu.ng

ABSTRACT

Over the past decades, humans as well as wildlife have been routinely exposed to different environmental pollutants such as crude oil, fertilizers, pesticides and pharmaceutical effluents, on a daily basis. This study was to investigate the adverse effects of Water soluble fractions(WSF) of crude oil on the reproductive performances of the Wistar albino rats (*Rattus norvegicus*). Thirty (30) mature Wistar rats (fifteen males and fifteen females) were randomly selected and paired so that a single male was paired with a single female for mating to occur. The gravid females were later selected into three (3) different groups, and were subjected to different concentrations (100%, 50%, and 0%) of the WSF of crude oil by oral administration. Results of this study showed that litter size was significantly affected by the WSF of the crude oil. Group A was significantly ($p<0.05$) lower than group B which was also lower than the control group .Birth weight was also significantly ($p<0.05$) lower in group A than group B which was also lower than the control group. Effects of the WSF of crude oil on the gestation period was not significant ($p>0.05$) different amongst the groups. Still birth was also not significantly affected by the WSF of crude oil among the test groups. Progesterone was significantly ($p<0.05$) affected by the WSF of crude oil between test groups when compared with the control group, Generally, the effect of the WSF of crude oil on the examined parameters was said to be dose- dependent. These findings suggested that exposure to WSF of crude oil affected the reproductive performance of the rats.
Keywords: Gestation length, still birth, Water soluble fractions of crude oil, progesterone

INTRODUCTION

Oil production is a big source of world energy, and revenue generation to oil producing economy. However, in addition to these huge advantages, short comings in form of negative environmental impacts that accompany oil production especially in the wetland ecosystem are also sources of concern [1] . Though while evaluating environmental impacts, the focus had mostly been on the ecosystem as it affects man economically and socially, with less consideration on the wild animals especially mammals and their physiological changes like changes in reproductive performances and the endocrine system etc due to this pollution [2] .This work is out to evaluate impact of water soluble fraction (WSF) of crude oil on reproductive performances of Wistar albino rats (*Rattus norvegicus*)

One of the major problems of the inhabitants of the Niger Delta region of Nigeria is the contamination of water and aquatic lives by crude oil. This contamination may not necessarily lead to outright mortality but may have significant effects on the physiologyof animals. The severity of the problems in the inhabitants of the area depends upon the route of administration of these pollutants.(oral, respiratory or skin contact). In this study oral administration was adopted, hence, the need for the preparation of different WSF of crude oil concentrations in this research. These pollutants have been implicated in many biochemical and toxicological effects on aquatic and terrestrial animals [3].

WSF of crude oil is that small fraction of the oil containing components sparingly or fully soluble in water [4] WSF of crude

oil comprises of toxic components such as polycyclic aromatics hydrocarbons (PAHs), mono-aromatics like benzene, toluene, xylene and ethyl-benzene; phenols and heterocyclic compounds containing sulphur, nitrogen and heavy metals [5]. Organisms exposed to WSF of crude oil take up the dissolved components and react to their effects.

Humans are routinely exposed to many environmental contaminants or pollutants intentionally or unintentionally, of which crude oil is one of the major environmental contaminants, or any of its fractions on daily basis. In sofar there is paucity of literature on the impact of ingestion of the WSF of crude oil or crude oil contaminated feed on reproduction and endocrine physiology of humans. Therefore, this study was to investigate the potential impacts of WSF of crude oil on reproductive performance (fecundity), using Wistar rats as a mammalian animal model. There is, therefore an urgent need to create awareness of the adverse effect of the WSF of crude oil on the maternal reproductive performance, especially inhabitants of oil rich rural areas.

MATERIALS AND METHODS

Collection of Samples

Fresh sample of crude oil used in this experiment was obtained from Center of Ecological Studies, University of Port Harcourt, Rivers State, Nigeria.

Preparation and Preservation of the WSF of Crude Oil

The WSF was prepared according to the method of [6]. This was done by adding one part of the crude oil to two parts of distilled water which was slowly mixed in a 2000ml volumetric flask. The flask was covered with aluminum foil, held tightly with rubber band to avoid evaporation of volatile components of the crude oil during stirring. This mixture was allowed to stir using magnetic stirrer for 24hours as recommended by [7] and also adopted by [8]. After that, the mixture was left to stand in a separating funnel for 12hours, then the lower, hydrophilic phase was withdrawn, and used as WSF of the crude oil.

It was collected in to a Winchester bottle as 100% stock. The total hydrocarbon content in the 100% stock was 0.352mg/ml. The stock was further diluted with distilled water to give 50% concentration of WSF, which was also stored in another Winchester bottle, and covered. The samples (100% and 50%) were stored at room temperature until required for use.

Experimental Animals

The thirty (30) Wistar rats used for this study were obtained from the animal house of the Department of Animal and Environmental Biology, University of Port Harcourt, Rivers State Nigeria. The rats were acclimatized for two weeks prior to the commencement of the experiment. These animals weighed between 185-200g, and were housed in rubber bowl cages, covered with wire gauze. They were fed commercially prepared feed and each animal consumed an estimated quantity of 35g of feed per day. Drinking water was also provided *ad libitum.*

Experimental Procedure

At the end of the period of acclimatization, fifteen female rats were selected randomly and separated. One male rat was paired with one female rat in each plastic bowl cage for mating to occur. Mating and pregnancy were assumed to have taken place by presence of sperm in vaginal fluid detected by vaginal smears. This was done by introducing about 0.1ml of 0.9% saline solution 2-3 times gently into the vagina of the rat to produce a vagina lavage. The pipette was withdrawn and its content was placed on a microscope slide and viewed using ×40 magnification lens of the microscope. There was also,a considerable weight gain; abdominal palpation confirmed positive for pregnancy. The gravid rats were then placed in three groups (two test groups (A and B) and the control (C)) at rats per group. They were all fed normal feed and water*ad libitum.* Group A and B wereorally administered 1ml of 100% and 50% WSF of crude oil respectively. Parameters measured include litter size, still birth, birth weight, gestation period and progesterone levels.

Sample Collection and Analysis

On the fourteenth day of gestation, blood samples were collected through tail

puncture using warm water and needle. The blood samples were introduced into clean dry lithium heparinized bottles and sent to the laboratory for female reproductive hormones analysis using the method described by [9].

Statistical Analyses

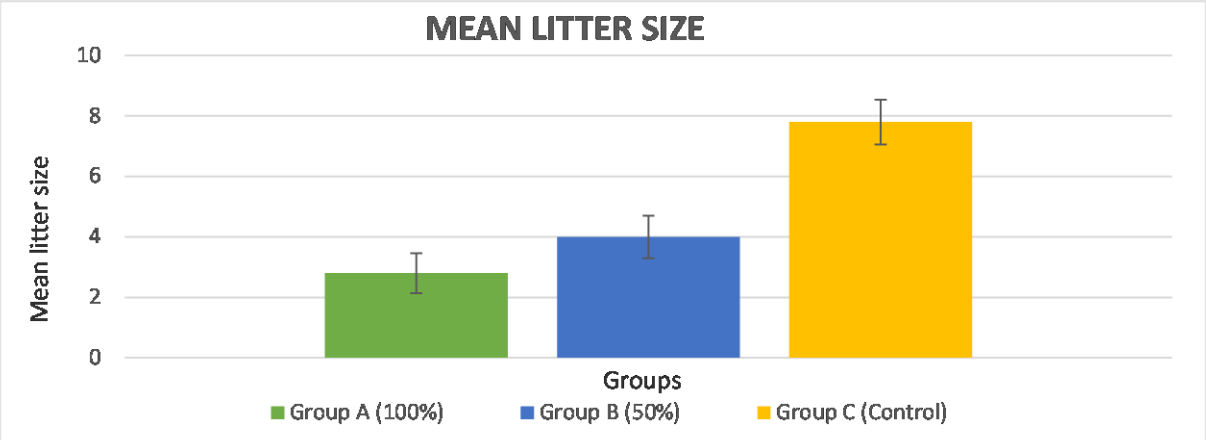
The results were expressed as mean ± standard error of the mean (SEM) and were analyzed using Analysis of Variance (ANOVA) in Complete Randomized Design (CRD). The data was further analyzed with Turkey for multiple comparisons between groups using the statistical package for social sciences (SPSS) software application version (20). The values of $p \leq 0.05$ were considered statistically significant.

RESULTS

The effect of the WSF of crude oil on reproductive performance was evaluated and each reproductive parameter presented in different bar charts.

The Effect of WSF of Crude Oil on Litter Size

The litter size of the various groups in figure 1 showed that control group had the highest litters with mean value of 7.8± 0.735 when compared with other groups. The group A (100%) and group B (50%) had values of 2.8±0.663 and 4±0.707 respectively. Litter size variation between the groups were statistically significant at ($p<0.05$).



The Effect of the WSF of Crude Oil on Birth Weight

The birth weight of the control with mean value of 7.206±0.31 was higher than the treatment groups, as showed in figure 2. The Group A and Group B had mean values of 5.004±0.26 and 5.452±0.23 respectively. The mean difference between groups were statistically significant at ($p \leq 0.05$).

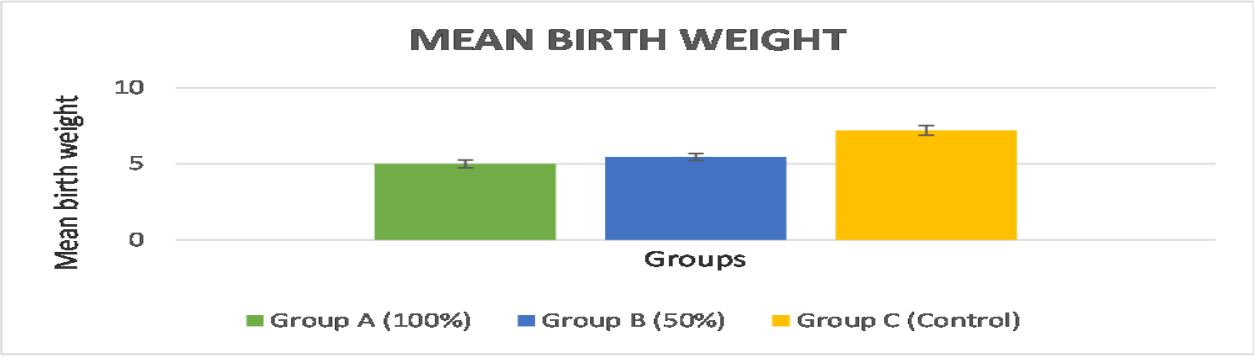


Figure 2: Effect of the WSF of crude oil on mean birth weight

The Effect of the WSF of Crude Oil on Stillbirth

The control group recorded no stillbirth, while group A recorded the highest still birth followed by group B as showed in the table 1 below. The effect of WSF of crude oil on stillbirth was not significantly different (p>0.05).

Table 1: Effect of the WSF of crude oil on still birth

Replicates	Group A (100%)	Group B (50%)	Group C (Control)
1	1	1	-
2	-	-	-
3	2	1	-
4	-	1	-
5	2	-	-
Total	**5**	**3**	-
MEAN±SEM	**1±0.44**	**0.6±0.25**	-

-No still birth

The Effect of the WSF of Crude Oil on Gestation Length

The gestation length of some of the animals in the test groups were affected, except the control group that had normal gestation length of 21 days though the differences were not statistically significant. (p>0.05) (figure 3). The administration of the WSF of crude oil altered the gestation length of some animals in the test groups, prolonging their gestation length, though the effect is not statistically significant

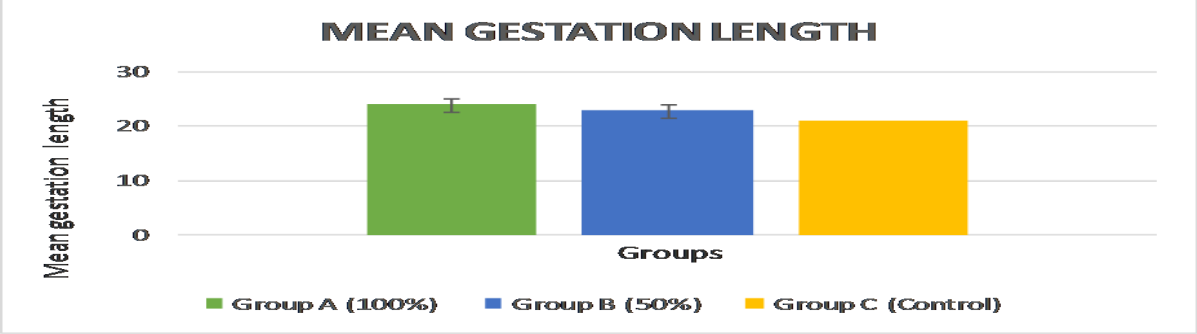


Figure 3: Effect of the WSF of crude oil on gestation length

The Effect of the WSF of Crude Oil on Progesterone Level

The progesterone was significantly affected by the WSF of crude oil between the groups as indicated in figure 4. Progesterone level significantly increased from Group A with the mean value of 5.9±0.633, Group B had 7.06±0.797, and Group C which is the control, had 9.12±0.368

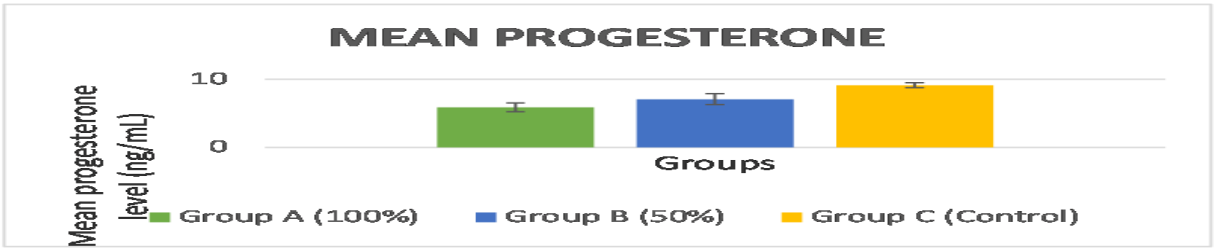


Figure 4: Effect of the WSF of crude oil on progesterone level

DISCUSSION

One of the major problems of inhabitants of Niger Delta region of Nigeria is the contamination of water and aquatic lives by crude oil spills. This contamination might not lead to direct mortality but may have a significant effect which can lead to physiological stress and dysfunction in animals. One of the physiological stresses being infertility, which has been a major clinical problem affecting people medically, psycho-socioeconomically, as well as family instability. In fact, infertility has been associated with the inhalation of environmental pollutants [10], and exposure to environmental pollutant- associated lifestyle and endocrine disrupting compounds [11].

The result of this study provided the information that exposure to the WSF of the crude oil by oral administration had adverse effects on the reproductive parameters examined. Moreover, other researchers had reported the toxic effects of the WSF of crude oil with association to abnormal values on Liver Function Test (LFT) [8] and also reduction in packed cell volume and increase in leukocyte count of the Wistar albino rats [12].

The mean litter size was significantly lower in treatment groups while compared to the control ($p<0.05$). This gives a vivid evidence of the effect on the reproductive system. Results also showed that it is dose-dependent reduction which was in agreement with [13] [14]. Prolonged gestation periods were observed in group A and group B with mean gestation lengths of 29 and 28 days respectively as compared with the control group. The gestation length was not significantly ($p>0.05$) affected by the WSF of the crude oil between the test groups. The reduction of birth weight in group A and group B when compared with the group C (control) could be probably due to variation observed in gestation period [15]

The values of mean stillbirth difference was observed to be dose-dependent, such that group A and group B had mean values higher than group C which had no recorded stillbirth. This observation was supported by the finding of [16]. Although the effect was not significant ($p>0.05$) when compared to the control. The effect of WSF of crude oil on the progesterone level was evaluated. Progesterone is a luteal hormone which is synthesized by the corpus luteum [17], the adrenal cortex and during pregnancy (by the placenta) [18]. It plays a vital role in the maintenance of pregnancy and has an antiestrogenic effect; blocking the growth of endometrium produced by estrogen [18].

The reports by [19] states that crude oil acts as an anti-androgenic compound, and there by inducing spontaneous abortion, still births and reproductive malfunction, following the ingestion of hexachlorobenezene (a component of crude oil) by the female rats. Data showed that there was significant ($p<0.05$) decrease in the progesterone level in the female test groups when compared to the control which is in accordance with the findings of [20]. The reduction in test groups progesterone maybe due to neurotoxic effect of crude oil components on the hypothalamus; that is involved in the production of hormones [21], [22], [23] or the formation of cystic spaces in the ovary; a pathological replacement of functional hormone producing cells and the formation of interfacing bundles of closely packed fibrocystic type cells [13]. The significant reduction in the progesterone level caused by the exposure to crude oil may be responsible for the observed fertility defects among the test group rats [20].

CONCLUSION

This study has demonstrated that exposure to WSF of crude oil affects the reproductive performance of the gravid Wistar albino rats. Also this study showed that ingestion of the WSF of crude oil interfere with secretion of the reproductive hormones, thus which will lead to irregularity in gestation length, still birth, birth weight.

RECOMMENDATION

From the results of this study, it is therefore suggested that humans, especially pregnant women, should be discourage from ingesting crude oil which is believed to be a curative agents to some diseases, which might lead to possible detrimental effects on both the reproductive and general performance of the affected individuals. Also there should be a close monitoring of the oil rich areas in case of oil spill on water bodies used for domestic purposes for immediate cleaning in order to safeguard the health of the inhabitants of the Niger Delta.

REFERENCES

1. Omoregie, E., Ufodike, B. C., & Onwuliri, C. E. (1997). Effect of Watwer Soluble Fractions of Oil on Carbohydrate Reseves of Oreochromis niloficus. *Journal of Aquatic Science, 12*, 1-7.
2. Olawale, O., & Onwurah, I. N. (2007). Preliminary inestigation into possible endocrine disrupting activity of Bonny light crude oil contaminated on Wistar rats. *Biochemistry, 1*, 23-28.
3. Ovuru, S. S., & Ekweozor, I. K. (2004). Haematological changes associated with crude oil ingestion in experimental rabbits. *African Journal of Biotechnology, 3*, 346-348.
4. Nwadukwe, F. O., Ayaobu-Cookey, I. K., & Matanmi, M. (2006). Performance of three age groups of Clarias gariepinus and F1 hybrid (C. gariepinus * Heterobranchus longifilus) fry in ponds. *African Journal of Zoology Environmental Biology, 8*, 140-149.
5. Saeed, T., & Al-Mutairi, M. (2007). Chemical composition of water soluble fraction of lead gasolines in sea water. *Enviro Int, 4*, 1117-119.
6. Anderson, J. N., Neff, J. M., & Cox, B. A. (2001). Characteristics of dispersions and water soluble extract of crude oils and their toxicity to estuarine crustaceans and fish. *Marine Biology, 1*, 75-88.
7. Parker, P. L., Winters, K., & Batherson, J. C. (1999). Effect and Sinks of Hydrocarbons in the Aquatic Environment. *American Institute Sciences*, 256-269.
8. Patrick-Iwuanyanwu, K. C., Okon, E. A., & Nkpaa, K. W. (2013). Hepatoxiclogical evalaution of water- soluble fraction (WSF) of crude oil in Wistar albino rats. *International Journal of the Nigeria Society for Experimental Biology, 25*, 17-22.
9. Radwanska, E. (1976). Plasma progesterone and estradiol estimations in menstruating infertile women. *Acta Eur Fertility*(7), 39-47.
10. Messenger, S. (2010). *Air pollution can harm human production.* Retrieved 2015, from www.treehugger.com
11. Akintunde , O. W., Ameen , S. A., & Olaniyan, L. W. (2012). Commonly used hydrocarbonsadversly affects male reproduction. *Veterinary Research, 5*, 8-12.
12. Igwebuike, U. M., Obidike, R. I., Nwankwo , I. O., & Okwechime, I. O. (2007). Effects of Nigeria Qua Iboe Brent crude oil on rat spleen and haematological parameterss. *Veterinary Archive, 2*, 247-258.
13. Nwafor, A., Asiegbu, L. N., Adienbo, M. O., & Mmom, F. C. (2013). Antifertility activity of ingestion of Nigeria Bonny Light Crude Oil contaminarted feed in male rat reproduction. *American Journal of Research Communication, 1*(2325-4076), 210-218.
14. Farombi , E. O., Adedara, I. A., Ebokaiwe, A. P., & Teberen, R. (2010). Bonny Light Crude Oil disrupts antioxidant systems in tested and sperm of rats. *Journal of Archives of Environmental Contamination and Toxicology, 59*, 166-174.
15. Raji, Y., & Hart, V. O. (2012). Influence of prolonged exposure to Nigeria Bonny light crude oil on

fertility indices in rats. *Nigeria Journal of Physiological Science, 27*, 55-63.

16. Nwaigwe, A. N., Anya , K. O., Nwaigwe, C. O., & Nwaigwe, C. U. (2012). The effect of Nigeria Qua-iboe Brent crude oil on reproductive performance of female Wistar albino rats. *Journal of Environmental Science and Technology, 5*, 84-90.
17. Agu, G. O. (2015). A Tribute to the Corpus Luteum; An Ephemeral Organ of Mammalian Reproduction. Port Harcourt: University of Port Harcourt Press Limited.
18. Rastogi , S. C. (2007). *Essentials of Animal Physiology* (4th ed.). New Delhi: New Age International Limited Publishers.
19. Alvarez, C., Alvarez, P., & Randi, A. (2000). Reproductive effects of hexachlorobenzene in female rats. *Journal of Applied Toxicology, 4*, 81-87.
20. Nwankwoala , R. N., & Gerogewill, O. A. (2001). The effect of exposure of Nigeria crude oil on Rats and Guinea pigs. *Journal of Medical Physiology, 6*, 57-58.
21. Indarto, D., & Izawa, M. (2001). Steriod Hormones and Endocrine disruptors: Recent advances in receptor-mediated actions. *Yonago Acta Medica, 44*, 1-6.
22. Asara, A. A., Tonkiri, A., & Eboh , A. S. (2014). The effects of acute exposure of Nigeria bonny light crude oil on the reproductive of pregnant Wistar rats. *Academia Journal of Scientific Reseach, 2*, 1-6.
23. Ogechukwu, O. J., Ajuluchukwu, N. A., Ifedioranma, O. S., & Onyemelukwe, A. O. (2012). Histopathological and hormonal disrupting effect of crude oil on the ovary of Chinchilla Rabbits. *Journal of Toxicology and Environmental Health Sciences, x*, 1-4.