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The Effect of Nigerian Qua-iboe Brent Crude Oil on the Reproductive Performance of Female Wistar Albino Rats

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ABSTRACT

The reproductive performance of rats given graded levels of the Nigerian Qua-Iboe brent crude oil was investigated in 32 female Wistar albino rats. The experiment was divided into three parts, whereby, crude oil was given orally at the dose of 0.1, 0.2 and 0.4 mL, corresponding to Groups A, B and C, respectively, for four weeks, while the untreated group served as control (Group D). Experiment one investigated the effect of the crude oil on the oestrous cycle in rats divided into four groups of four replicates, monitored for four consecutive cycles for regularity of oestrous cycle using vaginal cytology. Three of the four groups were drenched with graded levels of the crude oil while remaining group served as the control. Experiment two investigated the effect of crude oil on gestation, using another set of female Wistar albino rats which were grouped and drenched as in experiment one. Mating was monogamous, while drenching done every other day following mating. The gestation length, litter size, stillbirth and gross malformation of foetuses were monitored. The same group of animals in experiment two was used for experiment three, whereby the animals were drenched prior to mating and until parturition. Results showed irregularity of oestrous cycle in the drenched groups, with significant differences ($p<0.05$) existing between the experimental group and control group considering stillbirths, conception rates, gestation length and litter size. These findings suggested that exposure to Nigerian Qua-Iboe Brent crude oil affected the reproductive performance of the rats.

Key words: Oestrous cycle, gestation length, mating oral administration, vaginal cytology, still birth

INTRODUCTION

The Nigerian Qua-Iboe brent crude oil, mined by Exxon-Mobil in Akwa-Ibom state of Nigeria, is obtained from numerous off-shore fields in the Bight of Biafra in southeastern Nigeria. Often times, this substance causes very serious environmental pollution particularly during oil spillage, occurring as a result of activities of petroleum industry and pipe-line vandalization (Adeyemi, 2004). However, cases of misuse of this substance by Individuals abound, as it is known to be used liberally by some of the indigenes who believe that it can repel witches when applied either topically, or by oral administration on afflicted individuals, while some Nigerians and people

from other developing countries such as Kenya, Tanzania, Zimbabwe, Ghana and Tunisia depend on crude oil for unorthodox treatment of ailments such as stomach ache, diarrhoea, respiratory distress and convulsion (Dede *et al*., 2002).

Generally, various studies on crude oil have revealed that it has serious deleterious effects on soils (Erdogan and Karaca, 2011; Jeroh *et al*., 2011; Mary and Dolor, 2007), plants (Baek *et al*., 2004; Agbogidi *et al*., 2007), aquatic life (Ndimele *et al*., 2010; Daka and Ekweozor, 2004) and even on organisms such as the macrobenthic invertebrates (Arimoro and Adamu, 2008). However, humans and other animals are also adversely affected.

According to Otaigbe and Adesina (2005), studies in a two-year old female child showed pneumonitis, incoordination, shock, acute renal failure, intestinal obstruction, conjunctivitis and oesophagitis.

Aslani *et al*. (2000) reported bloody stools, coughing, constipation, infertility and sudden death in female goats exposed to West Texas intermediate crude oil while studies conducted by Igwebuike *et al*. (2007) revealed that exposure of male rats to Nigerian Qua-Iboe brent resulted in reduced packed cell volume, increased total leukocytes count and reduced cauda epididymal sperm reserves. This study, therefore, investigated the effect of administering the Nigerian Qua-Iboe Brent crude oil on the reproductive performance of female Wistar albino rats.

MATERIALS AND METHODS

A total of thirty two female Wistar albino rats (200-250 g) and sixteen male Wistar albino rats (250-270 g) were used for the work which was carried out between 2009 and 2010. The animals were supplied and housed by the Department of Obstetrics and Reproductive Diseases, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The test substance used in this study, the Nigerian Qua-Iboe Brent crude oil, was obtained from Exxon-Mobil, Akwa Ibom State of Nigeria.

Animal management and experimental design: This is a three-phased experiment whereby the animals were housed in the laboratory animal house of Department of Obstetrics and Reproductive Diseases, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, for four weeks. They were accommodated in metal cages and given feed and water ad libitum. At the end of this period and in the absence of disease, the first phase of the study started with sixteen female rats which were randomly selected and divided into four groups of four replicates. These animals were not mated and the oestrous cycle was monitored for four consecutive cycles for regularity before drenching with crude oil. The frequency of treatment was daily and graded levels of the crude oil were given as follows: Group A (0.1 mL), Group B (0.2 mL), and Group C (0.4 mL) while Group D was not drenched and served as the Control. The rats were drenched daily between 6.00 and 8.00 a.m. daily and their vaginal swabs collected between 1.00 and 2.00 p.m. to make vaginal smears for vaginal cytology which was used in monitoring the state of oestrous cycle.

For the second experiment, sixteen female albino rats were also randomly selected and also placed in four groups of four replicates. These animals were subjected to monogamous mating and drenching started after sighting of vaginal plugs and continued throughout the gestation period and until they gave birth. Parameters monitored were gestation length, stillbirth and gross malformation. Graded levels of crude oil were administered as follows: Group A (0.1 mL), Group B (0.2 mL), Group C (0.4 mL) and Group D (control).

For the third part of the experiment the same rats used in experiment two were used to investigate the effect of prolonged administration of the Nigerian Qua-Iboe Brent crude oil. The animals were re-subjected to monogamous mating and the frequency of treatment was every other

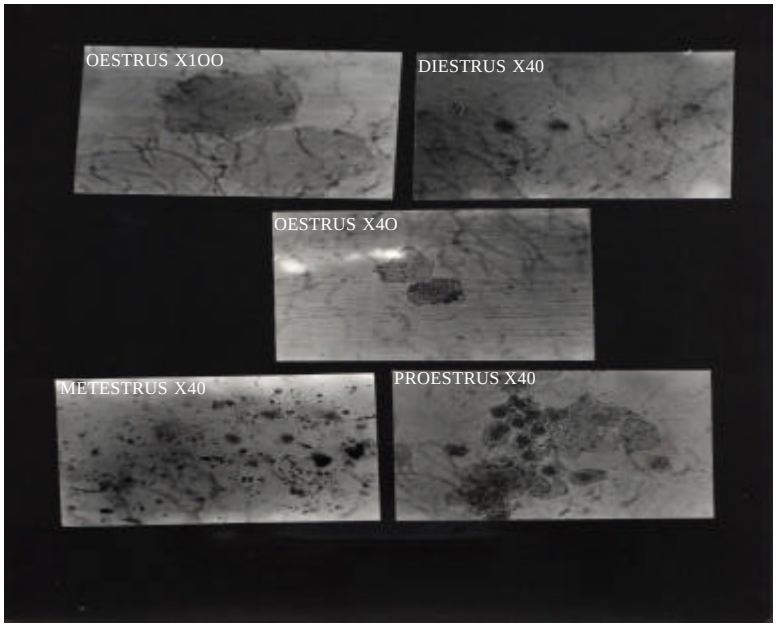

Fig. 1: Micrograph of vaginal cytology showing the different stages of oestrous cycle

day. The animals were drenched prior to mating and after sighting of vaginal plugs, drenching continued throughout the period of gestation and until parturition. The parameter monitored was conception rate.

Slide preparation for vaginal cytology: Vaginal cells were collected using a sterile swab stick moistened with normal saline for lubrication and introduced to the vagina to the depth of cotton head to avoid stimulation of the cervix which results in pseudo pregnancy. The smear was made by rolling the swab on the clean grease-free glass slides and allowed to air dry. Two drops of methanol from Pasteur pipette were placed on the smear to fix it for three minutes. After fixation, the Giemsa stain (10%) dilution was used to stain the slides and allowed to stand for ten minutes. After staining, the slides were washed with phosphate buffered distilled water and allowed to air-dry. The slides were thereafter viewed under the microscope at X40 and X100 objective lens (Fig. 1). The cells seen were used to denote the stage of the oestrous cycle. This was done for both the experimental and control groups.

Statistical analysis: The data obtained from this study were analysed using Analysis of Variance (ANOVA) in Complete Randomized Design (CRD) as described by Obi (2000). Means showing $p<0.05$ were considered statistically significant and were separated using Duncan's Multiple Eange Test (DMRT) (Steel and Torrie, 1980).

RESULTS

The effect of Qua-Iboe Brent on oestrous cycle, as monitored through vaginal cytology showed only one animal in group A to have a regular cycle, while the animals in groups B and C had irregular cycles. All animals in the Control group maintained regular cycles as shown in Table 1.

Table 1: The effect of Qua- Iboe Brent on oestrous cycle

Replicates	Group A (0.1 mL)	Group B (0.2 mL)	Group C (0.4 mL)	Group D (Control)
1	-	-	-	+
2	-	-	-	+
3	+	-	-	+
4	-	-	-	+

-:Irregular, +: Regular Oestrus

Table 2: The effect of Qua-Iboe Brent on the mean gestation length

Replicates	Group A (0.1 mL)	Group B (0.2 mL)	Group C (0.4 mL)	Group D (Control)
1	0	0	22	21
2	0	24	23	21
3	22	28*	28*	21
4	22	30*	0	21
Mean±SEM	22.00±0.00^{a}	27.33±1.76^{b}	24.33±1.86^{ab}	21.00±0.00^{a}

Means with similar superscripts in a row are not statistically significant at p≤0.05. *Prolonged gestation, SEM: Standard error of the mean

Table 3: The effect of Qua-Iboe Brent on the mean litter size

Replicates	Group A (0.1 mL)	Group B (0.2 mL)	Group C (0.4 mL)	Group D (Control)
1	0	1	2	7
2	4	0	0	9
3	9	3	4	6
4	8	9	1	10
Mean±SEM	5.25±2.01^{b}	3.25±2.0^{b}	1.75±0.85^{c}	8±0.91^{a}

Means with similar superscripts in a row are not statistically significant at p≤0.05

Table 4: The observed mean still birth in the various groups

Replicates	Group A (0.1 mL)	Group B (0.2 mL)	Group C (0.4 mL)	Group D (Control)
1	0	2	5	None
2	1	0	3	None
3	1	1	5	None
4	2	2	0	None
Mean±SEM	1.05±0.41^{b}	1.25±2.0^{b}	3.25±1.18^{a}	None

Means with similar superscripts in a row are not statistically significant at p≤0.05. 0: No conception

In Table 2, the gestation length of all the animals in the treatment groups were affected such that the animals in Groups B and C (0.2 and 0.4 mL) had prolonged gestation which ranged from 28 to 30 days (mean = 27.33 and 24.33, respectively). This showed that the administration of crude oil could significantly alter the gestation period in rats particularly at the levels administered. More so, their differed significantly (p = 0.05) from those of Group A (0.1 mL) and the Control, which however, did differ (p>0.05) significantly from each other.

The mean litter size of the various groups in Table 3 showed that the control had a significantly higher mean litter size compared to the treated groups. However, Group A had a higher mean litter size of (5.25±2.01) compared to groups B and C (3.25±2.0 and 1.75±0.85, respectively), although the mean litter size of Group A did not differ significantly (p>0.05) from Group B. This followed a similar trend in the observed mean still birth in the various groups as shown in Table 4, whereby the mean litter sizes of Groups A and B did not also differ significantly (p>0.05) compared to Group C (3.25±1.18). This showed that the mean litter size was affected as the quantity of crude oil administration increased.

Table 5: The Chronic effect of Qua-Iboe Brent on pregnancy

Replicates	Group A (0.1 mL)	Group B (0.2 mL)	Group C (0.4 mL)	Group D (Control)
1	-	-	-	+
2	-	-	-	+
3	+	-	-	+
4	-	-	-	+

+: Conception, -: No conception

The chronic effect of Qua-Iboe Brent on pregnancy (Experiment 3) in Table 5 indicated that conception occurred only in the third animal in Group A, after a prolonged drenching with 0.1 mL of the crude oil. Animals which received prolonged drenching with 0.2 and 0.4 mL (Groups A and B), respectively could not conceive while the control had normal conception and gestation. The conception noted in the third animal of Group A may be attributed to adaptation and/or tolerance, although the neonates of the treated group never survived beyond three weeks.

DISCUSSION

The results of this study provided the information that exposure to Nigerian Qua-Iboe brent by oral route had adverse effects on the parameters examined. However, some workers have reported on the toxic effects of crude oil with respect to abnormal values in the haematology of male rats of Wistar strain (Patrick-Iwuanyanwu *et al*., 2010) and reduced epididymal sperm reserve (Igwebuike *et al*., 2007).

The oestrous cycle in the treated groups was noted to be irregular compared to the control. However, failure to return to oestrous in this study may be attributed to interference of the crude oil with proper absorption of nutrients in the villi which subsequently may have affected the oestrous cycle. Consequently, Roberts (1971) reported that the nutritive state of an animal affects the oestrous cycle. He further observed that when rats lose about 15% of their body weight, in addition to loss of vital minerals such as phosphorus, cobalt, iron, copper, iodine and protein, their oestrous cycle may stop the as a result of impairment of secretion of the gonadotropic hormones. Prolonged gestation was observed in groups B and C respectively, with the mean gestational lengths of 28 and 30 days. This was statistically significant ($p<0.05$) compared to either group A or the Control which had non-statistically significant ($p>0.05$) mean gestational lengths of 22 and 21 days respectively (Table 1). The mean litter size was significantly high in the Control group ($p<0.05$), while in group C, which received the highest dose of crude oil had the lowest mean litter size of 1.75±0.85. The differences observed in the mean stillbirth was also suspected to be dose-dependent such that groups A and B had significantly lower means compared to group C, while the Control group had none. This observation is supported by the reports of Khann and Line (2005), who stated that petroleum crude oil contained substances such as Benzene, Toluene and Xylene which can be harmful to both fetuses and neonates.

Although, pregnancy was noted to occur in all the groups but some animals in the treated groups failed to conceive. However, conceptions noted in groups A (0.1 mL), B (0.2 mL) and C (0.4 mL) may be attributed to adaptation and/or tolerance. Conception failure may be attributed to interference with cleavage and implantation or failure to return to oestrus and ovulation. Moreso, is important to note that neonates of the treated groups were weak and never survived beyond three weeks. This result suggests that in the failed groups, the crude oil probably may have interfered with cleavage and implantation or caused failure of the rats to return to oestrus. It may have also interfered with secretion of the hormone of pregnancy-progesterone.

This assertion agrees with the works of Mcfarland (1984) who disclosed that the degree of petroleum toxicity is dependent on the dose, duration of exposure and frequency of administration. This study also supported the observations of Franklin and Lloyed (1982) who also reported foetotoxic effects of three American crude oil types namely-Prudehoe hills, Lost Hills Light and Belridge Heavy crude oils. This study thus, indicated that crude oils may actually have foetotoxic components as was elucidated in Experiment three, showing that prolonged use of Nigerian Qua-Iboe brent caused conception failure.

CONCLUSION

This study has demonstrated that exposure to the Nigerian Qua-Iboe brent affected the reproductive performance of the female Wistar albino rats and that the degree of crude oil toxicity may be related to both the frequency and dose of administration of the substance. However, other factors such as duration of exposure, adaptation and species of animal might interfere with the degree of toxicity. There is also the possibility that crude oil ingestion could interfere with secretion of reproductive hormones, thus, leading to impaired or irregular cycles. From the results of this study, it is therefore suggested that humans should be discouraged from ingesting crude oil which implies possible detrimental effects on both the reproductive and general performance of the affected individuals.

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