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ARTICLE

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

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SUMMARY

The presumptive effects of exposure to Nigerian Qua-Iboe brent crude oil on the reproductive performance of rats was investigated using 32 female Wistar albino rats. The experiment was conducted in three parts using graded levels (0.1ml, 0.2ml and 0.4ml) of Nigerian Qua-Iboe brent, administered orally for four weeks. The grades were designated A, B and C respectively. A fourth group which was untreated with Nigerian Qua-Iboe brent and designated group D served as control. Experiment one investigated the effect of the crude oil on the oestrous cycle in rats using vaginal cytology. Experiment two investigated the effect of crude oil on gestation, using the gestation length, litter size, stillbirth and gross malformation of foetuses as cardinal parameters. The same group of animals in experiment two was used for experiment three, which assessed the effect of prolonged administration of Nigerian Qua-Iboe brent on the conception rate of the treated groups. Results showed that the administration of Nigerian Qua-Iboe brent affected the oestrous cycle, gestation length, the mean litter size, and also caused still births in the drenched groups, particularly in a dose- dependent manner, while prolonged administration of Nigerian Qua-Iboe brent affected the conception rates in the treated groups compared to the control.

KEY WORDS: Nigerian Qua-Iboe Brent crude oil, Reproductive performance, Oestrous cycle.

INTRODUCTION

Nigerian Qua-Iboe brent crude oil is mined by Exxon –Mobil in Akwa-Ibom state of Nigeria and is known to be used liberally by the indigenes because of the belief that it can repel witches (Dede *et al*., 2001). In recent times studies on the crude oil have revealed that exposure of male rats to Nigerian Qua-Iboe brent resulted in some pathologies such as reduced packed cell volume, reduced cauda epididymal sperm reserves; however,a positive effect marked by increased leucocytic count was also observed (Igwebuike *et al*., 2005).

Generally, various studies on side effects of crude oil showed pneumonitis, incoordination, shock, acute renal failure, mechanical intestinal obstruction, conjunctivitis and oesophagitis in a two- year old female child (Otaigbe *et al*., 2005). Dede *et al*. (2001) also reported that crude oil distillates of Nigerian bonny light such as gasoline and kerosene substantially increased aspartate transaminase, alanine transaminase and alkaline phosphatase levels in rats exposed by oral routes. Aslani (2000) reported bloody stools, haemochezia, coughing, constipation, infertility and sudden death in female goats exposed to West Texas intermediate crude oil.

More so, Petroleum has been reported to elicit multiple types of toxic effects which include acute lethal toxicity, sub-lethal chronic toxicity or both, depending on the exposure, dosage and organs exposed (Orisakwe *et al.,* 2004). The desire to embark on this study was motivated by the alarming rate at which Nigerians and people from other developing countries such as Kenya, Tanzania, Zimbabwe, Ghana and Tunisia depend on crude oil for unorthodox treatment of certain ailments such as stomach ache, diarrhoea, respiratory distress and convulsion, as reported by Dede *et al*. (2001).

This paper therefore investigated the effects of Nigerian Qua-Iboe Brent crude oil on the reproductive performance of female Wistar albino rats.

MATERIALS AND METHODS

A total of thirty two (32) female Wistar albino rats (200-250g) and sixteen male Wistar albino rats (250-270g) were used for the work. 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 work, the Nigerian Qua-Iboe Brent crude oil, was obtained from Exxon-Mobil, Akwa Ibom state, Nigeria.

Animal management and experimental design

The animals were housed in the laboratory animal house for four weeks where they were given feed and water *ad libitum* and were accommodated in metal cages. Within this period their physiological parameters were monitored.

Experiment One

Sixteen (16) female rats which were randomly selected and divided into four groups of four replicates (A, B, C and D) were not mated and the oestrous cycle was monitored using the vaginal cytology technique for four consecutive cycles for regularity before drenching with crude oil. They were later drenched daily with graded doses of the Nigerian Qua-Iboe Brent crude oil by oral administration using the technique as follows: Group A 0.1 ml, Group B 0.2ml, Group C 0.4ml while Group D was not drenched and served as the control. Drenching was daily between 6.00am and 8.00am. Vaginal swabs were collected between 1.00pm and 2.00pm daily. The swabs were used to prepare vaginal smears for vaginal cytology, whereby the predominant vaginal cells were identified for monitoring the state of oestrous cycle.

Experiment Two

Sixteen (16) female albino rats were randomly selected and placed in four groups of four replicates as in Experiment one. Each female rat was paired with a particular male for mating (monogamous mating) and drenching (as in one above) started after sighting of vaginal plugs. This continued daily throughout the gestation period and until parturition. The gestational length of each female rat was monitored after mating, while at parturition, the rats were observed for stillbirth and gross malformations.

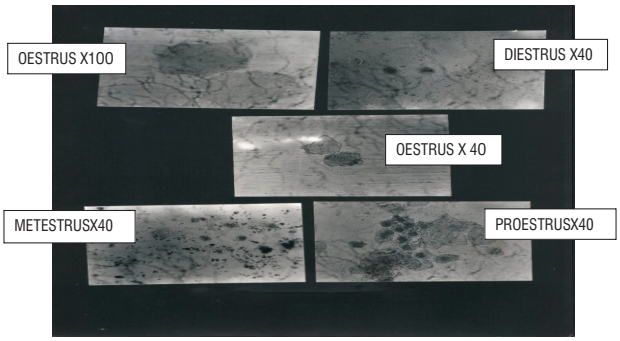
Experiment Three

For the third part of the experiment the same rats in experiment two were used to further investigate the effect of prolonged administration of the Nigerian Qua-Iboe Brent crude oil. They were drenched on alternate days prior to mating, and then re-subjected to monogamous mating. Drenching continued even after sighting the vaginal plugs and throughout the period of gestation. The conception rates of the animals in the treatment groups were monitored compared to the control.

Slide Preparation for vaginal cytology

Vaginal cells were collected using a sterile swab stick moistened with normal saline for lubrication. The swab stick was introduced into the vagina, to the depth of cotton head, to avoid stimulation of the cervix which could result 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. The cells seen were used to denote the stage of the oestrous cycle. This was done for both the experimental and control groups.

Micrograph of vaginal cytology for the different stages of oestrous cycle.



Statistical Analysis.

The data obtained were analysed using analysis of variance (ANOVA) in Complete randomized design (CRD). Means showing $P<0.05$ were considered significant.

RESULTS

Table I. The effect of Qua- Iboe Brent on oestrous cycle

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

- = Irregular ; + = Regular Oestrus

Table II. The effect of Qua-Iboe Brent on gestation length

Replicates	Group A 0.1ml	Group B 0.2ml	Group C 0.4ml	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]

0 indicates conception failure. * indicates prolonged gestation.
Means with similar superscripts in a row are not statistically significant at P≤0.05.

Table III. The effect of Qua-Iboe Brent on the mean litter size

Replicates	Group A (0.1ml)	Group B (0.2ml)	Group C (0.4ml)	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.
0 indicates conception failure.

Table IV. The observed mean still birth in the various groups

Replicates	Group A (0.1ml)	Group B (0.2ml)	Group C (0.4ml)	Group D (Control)
1	0	2	5	-
2	1	0	3	-
3	1	1	5	-
4	2	2	0	-
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 indicates conception failure, indicates no still birth.

TABLE V. Chronic effect of Qua-Iboe Brent on pregnancy

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

+ = Conception ; - = No conception.

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

In Table II, 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 (0.1ml) and the Control, which however, did differ (P> 0.05) significantly from each other.

The mean litter size of the various groups in Table III 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 IV, 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.

The chronic effect of Qua-Iboe Brent on pregnancy (Experiment 3) in Table V indicated that conception occurred only in the third animal in Group A, after a prolonged drenching with 0.1ml of the crude oil. Animals which received

prolonged drenching with 0.2ml and 0.4ml (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 epidydimal sperm reserve (Igwebuike *et al*., 2007).

The oestrous cycle in the treated groups showed different levels of irregularity which tended to increase with the dose of administered. 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 percent 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 Band 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 I). 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 (0.1ml), B (0.2ml) and C (0.4ml) 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(198_Hlt297729278_Hlt297729278 4) 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) 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 possible 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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