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The Impact of Crude Oil on Reproduction in Wistar Rats.

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ABSTRACT

Most rural communities of the Niger Delta rely on river water for drinking, but due to numerous oil spillages, these rivers have become polluted with crude oil. This research was designed to determine the possible effect of crude oil on reproduction in Wistar rats. A total of fifty virgin Wistar rats weighing on the average 185g were involved in this experiment and were divided into five groups(A,B,C,D and E), each having ten rats made up of five males and five females. In each group male and female rats were housed separately. In group A, the male rats were treated with 60mg/kg/day of crude oil dissolved in drinking water after and normal rat chow ad libitum, the female rats received tap water and rat chow ad libitum. In group B, the female rats were treated with 60mg/kg/day of crude oil dissolved in drinking water and normal rat chow ad libitum, the male rats received tap water and rat chow ad libitum. In group C, the male rats received crude oil contaminated as drinking water and were normal rat chow ad libitum, the female rats received tap water and rat chow ad libitum. In group D, the female rats received crude oil contaminated as drinking water and normal rat chow ad libitum, the male rats received tap water and rat chow ad libitum. Group E served as control and were fed normal rat chow and water ad libitum. Treatment lasted for seven weeks after which the rasts were allowed to mate, the female rats were examined in the morning for sperm plugs and this was used to determine the first day of pregnancy. After mating the male rats were sacrificed semen analysis was done and testes was harvested for histology, while female rats were maintained with tap water and rat chow throughout the period of pregnancy after which the liter size and liter weight, the gestation period and number of surviving pups after one week were noted the female rats were then sacrifice and ovaries harvested for histology. Data obtained was analyzed using the student's t test and ANOVA. Result showed significant ($p<0.05$) reduction in sperm count and motility of treated groups. There was also significant ($p<0.05$) reduction in liter size, liter weight and percentage pup loss in treated groups. Histological findings revealed distortions in the architecture of the testes and ovaries of treated rats. We conclude therefore that crude oil adversely affected reproduction in wistar rats.

Keywords: Crude oil, fecundity, reproduction.

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INTRODUCTION

Nigeria is the largest producer of crude oil in Africa and the 11th largest in the world, a major supplier to Europe and 5th largest supplier to USA in 2002. The southern part of the country especially the Niger Delta region provides most of the space for exploration and exploitation of crude oil. This region has many rivers and streams through which freshwater empties into the Atlantic Ocean. These rivers most of the time provide the only sources of drinking water and marine food for the local communities within the region. During exploration, exploitation and refining of crude oil, accident in the form of spillage often occurs resulting in the contamination of the environment, especially these sources of drinking water, exposing the inhabitants of these communities to danger. Generally, it has been estimated that an average of 11-54mg/L of the oil is dissolved in our coastal waters. The continuous existence of man depends on his ability to reproduce itself and his reproductive capacity is very much affected by his environment. We therefore carried out this study to assess the possible effect that exposure to crude oil may have on reproduction in wistar rats with emphasis on sperm count and motility in male rats, fecundity in male and female treated rats and histological changes in testis and ovaries associated with crude oil exposure [1-5].

MATERIALS AND METHOD

Wistar rats were obtained from the animal house of the faculty of basic medical sciences Ambrose Alli University Ekpoma and were tranfered to the animal house of the faculty of basic medical science Delta State University Abraka. They were housed under normal room conditions of temperature and humidity and were allowed a twelve hours light and dark cycle.

Bonny light crude oil was obtained from the petroleum training institute Warri Delta State Nigeria and crude oil contaminated water was obtained from Amukoko River in Sapele Delta State Nigeria.

Experimental Protocol

A total of fifty virgin wistar rats weighing on the average 185g were involved in this experiment and were divided into five groups(A,B,C,D and E), each having ten rats made up of five males and five females. In each group male and female rats were housed separately. In group A, the male rats were treated with 60mg/kg/day of crude oil dissolved in drinking water after considering it's LD_{50} of 5.96kg/kg^2 and normal rat chow ad libitum, the female rats received tap water and rat chow ad libitum. In group B, the female ratswere treated with 60mg/kg/day of crude oil dissolved in drinking water and normal rat chow ad libitum, the male rats received tap water and rat chow ad libitum. In group C, the male rats received crude oil contaminated as drinking water from polluted stream and were normal rat chow ad libitum, the female rats received tap water and rat chow ad libitum. In group D, the female rats received crude oil contaminated as drinking water and normal rat chow ad libitum, the male rats received tap water and rat chow ad libitum. Group E served as control and were fed normal rat chow and water ad libitum. Treatment lasted for seven weeks after

which the rasts were allowed to mate, the female rats were examined in the morning for sperm plugs and this was used to determine the first day of pregnancy [4].

After mating the male rats were sacrificed semen analysis was done and testes was harvested for histology, while female rats were maintained with tap water and rat chow throughout the period of pregnancy after which the liter size and liter weight, the gestation period and number of surviving pups after one week were noted the female rats were then sacrifice and ovaries harvested for histology. Litter size was obtained by counting the number of litter per female rat after delivery[3].Litter weight was obtained by weighing each pup with a digital weighing balance. Percentage pup lost in a week was obtained by counting the number of pup lost per female rat after one week and reporting it as a percentage of the total number of delivered by the same rat. Gestation period was determined by counting the number of days between conception (day of observed sperm plug) and delivery. Histological tissue preparation will be done by Standard histological methods using H and E technique Semen analysis was done using the new improved neuber counting slide. Data obtained were analyzed using student's t test and one-way ANOVA.

Ethical Approval

Ethical approval was obtained from the Delta State University College of health sciences ethical committee.

RESULTS

Table 1: Fecundity analysis for male experiment

Groups	Liter Size	Liter Weight (9)	Gestation Period(days)	Number Lost Per Week	% Lost Per Week
Control (group E)	9.1(1.8)	12.2(3.2)	21.4(0.5)	1.43(0.9)	14.2
Crude oil (group A)	3.3(2.0)* (p=0.01)	5.1(2.3I)* (p=0.01)	21.6(1.6)	2.0(1.2)	60.6
Contaminated H_20 (group C)	8.1(1.1)	7.5(1.2)* (p=0.05)	21.0(0.8)	4.1(2.6)	50.6

The results are presented in means and standard deviations and %, (n=5).
* Means significant compared to control values.

Table 2: Fecundity analysis for female experiment

Groups	Liter Size	Liter Weight (9)	Gestation Period(days)	Number Lost Per Week	% Lost in 1st Week
Control (group E)	9.1(1.8)	12.2(3.2)	21.4(0.5)	1.43(0.9)	14.2
Crude oil (group B)	6.3(3.1)* (p=0.05)	6.2(2.3)* (p=0.01)	21.1(0.8)	3.3(1.2)	52.4
Contaminated H_2O (group D)	6.1(3.3)* (p=0.05)	6.3(3.0)* (p=0.01)	22.0(1.2)	4.2(1.6)	68.9

The results are presented in means and standard deviations and %, (n=5).

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Table 3: Changes in sperm count following exposure to crude oil and diesel in rats

	Control (group E)	Crude treated (group A)	t-cal	p-value	R
Sperm count(million)	24.9(4.0)	18.22 (0.6)	2.83	P= 0.01	*

The results are presented in means and standard deviations (n=5).

Table 4: Sperm motility following exposure of male rats to crude oil

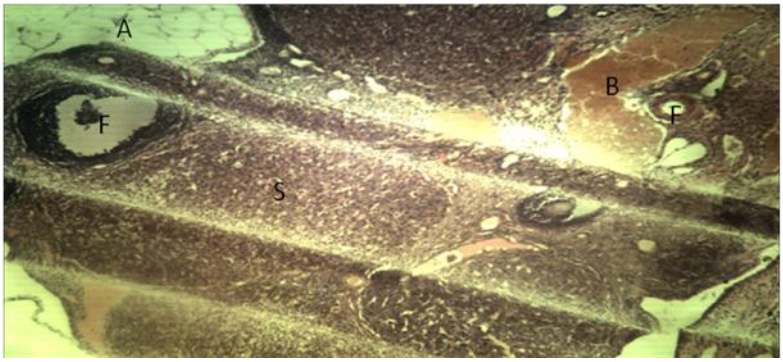
	Poor motility %	Good motility %
Control (group E)	7.0	93.0
Crude treated (group A)	18.6	81.4

Results show reduced motility in crude oil treated rats

Histological Analysis

Plate 1

The photomicrograph of ovary of control rat after histological preparation and staining with Hand E is presented (Plate 1).



Control Ovary (mag ×100)

Section of Ovary show several variable sized cystic gland lined by one or more layers of collumnar epithelial cells. Also seen are several follicular cyst surrounded by granulosa and theca cell. The background stroma is fibrocollegenous.

KEY: B= blood vessel, F=follicle, A= Adipocytes, H= Hemorrhage

Plate 2

The photomicrograph of ovary of contaminated water treated rat after histological preparation and staining with Hand E is presented (Plate 2).

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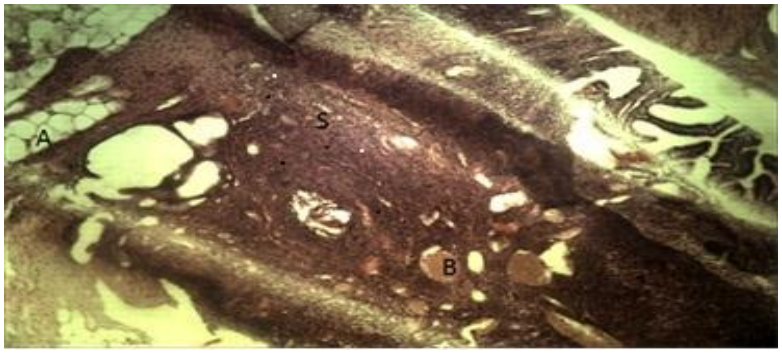


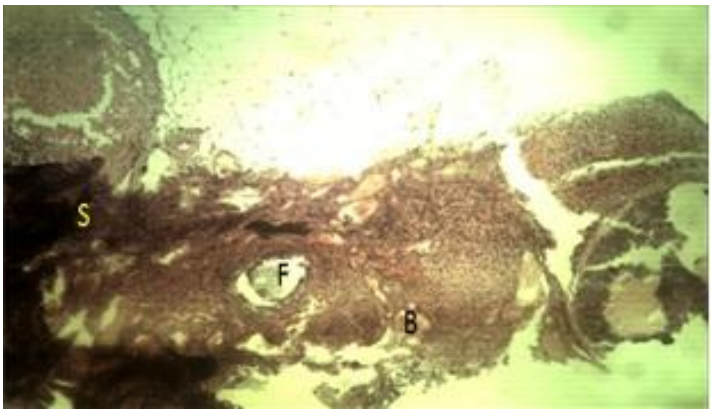
Contaminated water treated (mag ×100)

Section of Ovary show marked replacement of normal ovarian stroma with fibroblast cell arranged in bundles with thin interconnecting collagenous stands. No follicular arrangement is seen.

KEY: B= blood vess:el, F=follicle, A= Adipocytes, H= Hemorrhage

Plate 3

The photomicrograph of ovary of crude petroleum oil (60 mg/kg of bwt) treated rat after histological preparation and staining with Hand E is presented (Plate3).



Crude Oil Treated Ovary (mag ×100)

Section of Ovary show follicles composed of granulosa and theca cells with outer fibroblast. Some glands are cystically dilated and lined by collumnar cell. Some stroma cells have started undergoing necrosis.

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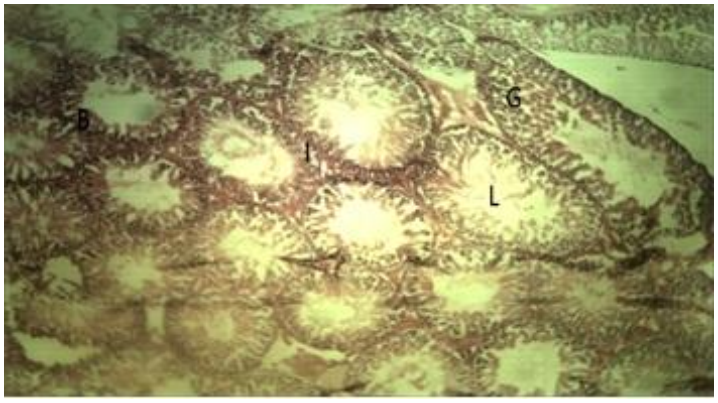
Table 5: Summary of severity of structural damage in sections of the ovary

Groups	Severity of structural damage
Control	Nil
Contaminated water	+
Crude petroleum oil	+ +

Key: +=30%,++=60%

Plate 4

The photomicrograph of testes of control rat after histological preparation and staining with Hand E is presented (Plate 4)



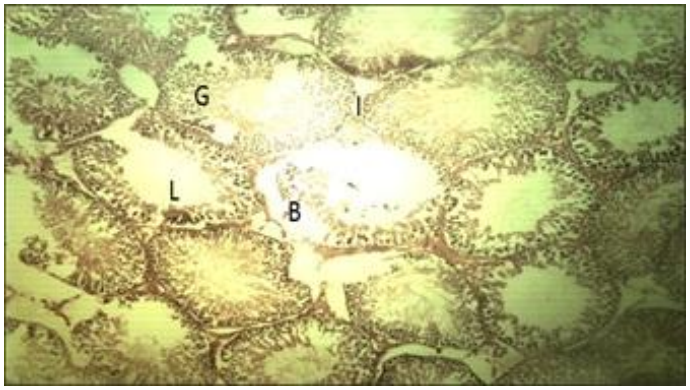
Control testis (mag ×100)

Section of testis showing variably sized seminiferous tubules with spermatocytes at different levels of maturation in the lumen. The surrounding interstitial connective tissue is cellular. A few blood vessels are also seen in the interstitium.

KEY: B= Basement membrane, G= Germinal epithelium, I= Intertisium, L=Lumen

Plate 5

The photomicrograph of testes of crude petroleum oil treated (60mg/kg of bwt) treated rat after histological preparation and staining with Hand E, is presented (Plat 5).



Crude oil Treated Rat Testes (mag ×100)

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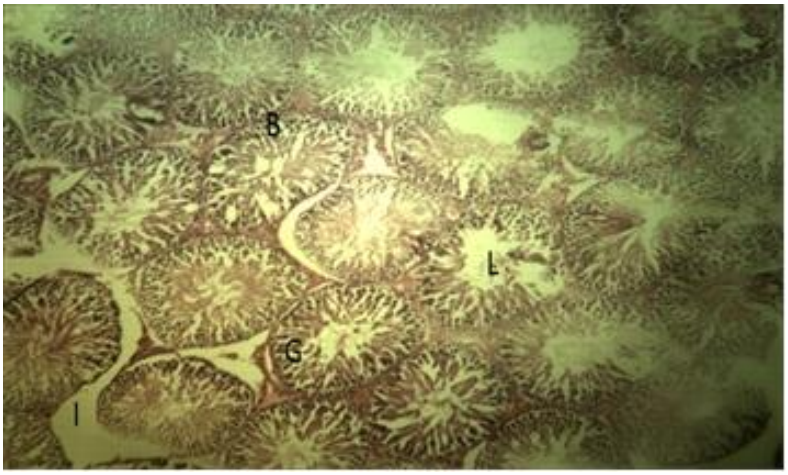
Section shows substantial loss of both seminiferous tubules and surrounding interstitial cells. There is obvious marked sperm cell maturation arrest. No matured spermatocyte is seen.

Plate 6

The photomicrograph of testes of contaminated water treated rat after histological preparation and staining with Hand E, is presented (Plat 6).



Contaminated water treated testes (mag ×100)

Section of testis is same as in plate 4.1 above, with loss of few seminiferous tubules however.

Table 6: Summary of severity of structural damage in sections of the testes

Groups	Severity of structural damage
Control	Nil
Contaminated water	+
Crude petroleum oil	+ +

Key: +=30%, ++=60%

DISCUSSION

The study reveals a significant reduction in liter size and liter weight in crude oil treated male and female experiments, however more severe in the female experiment. There was also significant reduction in sperm count and motility in male rats treated with crude oil. Histological study revealed degenerative changes in testis of rats treated with crude oil, and contaminated water (Disruption of basement membrane, increase in interstitial space with reduced cellularity and sperm maturation arrest). Major changes found in the ovaries of rats treated with crude oil, and brackish water is those of increase fibrosis and fatty changes. Histological changes in the testis are in agreement with jing-ying Huang et al., Who observed degenerative changes in rats exposed to motorcycle exhaust and Ebenezer et al., who reported severe congestion of interstitial vessels, decreased germinal epithelium, and increased number of vacuolization in testes of rats treated with Bony light crude oil. These degenerative changes may explain the reduction in fecundity observed in the male treated rats in this research. Our observation of reduced sperm count

and motility in rats treated with crude oil is also in agreement with jing-ying Huang et al., [5] and Ebenezer et al [6].

Major changes found in the ovaries of rats treated with crude oil, and brackish water are those of increase fibrosis and fatty changes. This may impact negatively on follicular growth and ovulation which may be responsible for the reduction in fecundity of treated female rats. We have not found similar reports on the effect of crude oil on the histology of the ovaries. The significant reduction in liter size, liter weight and percentage number of pups lost within the first week observed in female rats treated with crude oil and contaminated water may be as a result of passage of crude oil through the placenta as Khan et al., [7] reported that crude oil can pass through the placenta barrier and this may explain our findings. Our observed reduction in fecundity is in agreement with Olawale et al., [8]. This provides empirical evidence that crude oil actually interfere with reproduction in Wistar rats. This clearly shows that ingestion of crude oil contaminated water affects the reproduction of male wistar rats by reducing sperm count and motility and female rats by affecting ovarian function and transplacenta affectation of the of the fetus.

CONCLUSION

Oral intake of water contaminated with crude oil adversely affected the reproduction of wistar rats.

Acknowledgement

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