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Influence of prolonged exposure to Nigerian Bonny light crude oil on fertility indices in rats

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Summary: The effect of Bony Light Crude Oil (BLCO) on some sperm functions during short term exposure has been reported. The present study investigated the effect of long term ingestion of BLCO on fertility indices in male and female albino rats. Adult male rats in groups of five were exposed daily to 0, 200, 400 and 800 mg/kg BLCO (orally, p.o) dissolved in olive oil which served as the control, for six weeks. The male rats were cohabited with untreated adult female rats (for mating) during the last 6th week of treatment. Similarly, adult female rats in groups of five were exposed daily to 0, 200, 400 and 800 mg/kg BLCO (p.o) for 6 weeks. The rats were mated with untreated male rats during the last 6th week of treatment. Body weights of the treated rats remain unaffected. However, a significant decrease ($p< 0.05$) in the weight of the testis, epididymis, cauda epididymal sperm count, motility, viability and normal morphology of the spermatozoa was observed. There was 100% mating success as all untreated female rats with a significant decrease ($p<0.05$) in number, birth weight and survival rate of offspring delivered. BLCO treated female rats had irregular oestrous cycle with increased frequency of oestrous and metestrous phases and a decrease in the diestrous phase. A dose-dependent reduction in fertility success, number and birth weight of offspring of the BLCO treated females mated with normal males was recorded. Histological study of the epididymis and testis showed BLCO treatment-related lesions. The results suggest that, Bonny light crude oil reduced fertility indices of male and female rats.

Keywords*:* Bonny Light Crude Oil; Fertility, Male, Female, Rat

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INTRODUCTION

Human beings are routinely exposed to many environmental pollutants such as pesticides, fertilizers, crude oil or any of its fractions on a daily basis. These pollutants have been implicated in many biochemical and toxicological effects on aquatic and terrestrial animals (Ovuru and Ekweozor, 2004).The substances can bio-accumulate in food chains and disrupt biochemical or physiological activities of many organisms, thus causing carcinogenesis of some organs, mutagenesis in the genetic material and impairment in reproductive capacity in exposed population. Many chemical substances have been demonstrated to interfere with normal reproductive processes in several animal species (Indarto and Izawa, 2001).

One of such substances is Bonny Light Crude Oil (BLCO) which is a complex mixture of many different components. Crude oil, refined petroleum products, as well as polycyclic aromatic hydrocarbons are ubiquitous in various environmental compartments. Its exploration and transportation has generated a lot of environmental problems, especially in developing nations. It is noteworthy, that the devastating consequences of crude oil spill in the Niger Delta of Nigeria pose great hazards on both aerial and terrestrial environments. Although, limited exposure to crude oil may also occur during drilling, transporting and refining, accidental spillage accounts for a more serious exposure to crude oil by wildlife and humans (Adesanya et.al, 2009). Furthermore, many of the people who live in the oil rich areas are exposed to water from streams and ponds that have been polluted by oil spillage. This water is used for domestic activities such as drinking, cooking and washing by rural dwellers in the various oil rich areas of the Niger Delta in South-South Nigeria. It is important to note that, majority of the people in the communities also ingest crude oil either directly as curative agents for anti-poisoning (snake venom antidotes), anti-convulsion, treatment of skin infection or indirectly by eating marine animals found in surrounding coastal waters as a source of protein (Dede et al., 2002). BLCO is used in combination with olive oil in folklore medicine in some parts of

the Niger Delta region of Nigeria to treat burns, gastrointestinal disorders, ulcers, witchcraft attacks and poisoning (Orisakwe et al., 2000; Dede et al., 2002).

Although, various studies have been carried out on crude oil, very few have been directed at its impact on reproductive system. Increasing concern about the possible declining trend in fertility of man and wildlife animals over the past decades as a result of exposure to various environmental pollutants such as estrogenic agents and aromatic hydrocarbons have been widely reported (Lyons et al., 1999; Izegbu et al., 2005; Shittu et al., 2007; Shittu et al., 2008). Previous studies showed that BLCO significantly reduced sperm count, sperm motility and normal morphology within seven days of administration (Oriskwe et al., 2004; Adesanya et al., 2009; Farombi et al., 2010). Literature is scarce on whether or not this effect is capable of affecting fertility indices of these rats. The spermatogenic cycle of rats is about 10-14 days as reported in several studies (Leblond and Clermont, 1952; Clermont 1972; Johnson et al., 1980; Sinha et al., 1985; Laura et al., 1993; Breed and Taylor, 2000; Peirce and Breed, 2001). Moreover, there is a dearth of information on the effects of BLCO on female reproduction. The present study was therefore designed to investigate the effects of Bonny Light Crude Oil ingestion on fertility indices of male and female albino rats during long term exposure.

MATERIALS AND METHODS

BLCO: BLCO was obtained from the Nigerian National Petroleum Corporation (NNPC) Warri, Nigeria. The crude oil was diluted in olive oil according to previous studies (Dede et al., 2002; Owu et al., 2005) and served as the control in this study.

Animals: Adult male and female Wistar albino rats (weighing between 180-250g and aged about 10 weeks) obtained from the Central Animal House, College of Medicine, University of Ibadan, Ibadan Nigeria were used for this study. The animals were housed in wire mesh cages and kept in an environment of 12 hours dark and 12 hours light cycle at room temperature. They were allowed to acclimate to laboratory conditions for two weeks with feeds and water provided *ad libitum* prior the commencement of the experiments. Vaginal smears were taken daily, and only animals displaying at least two consecutive 4-day estrous cycles were used. All animals were observed for clinical signs of drug toxicity (such as tremors, weakness, refusal of feeds, diarrhea, weight loss, hair-loss, coma and death) throughout the duration of the experiment. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002).

Experimental protocol: The study was divided into two parts as follows:

Male fertility study: Twenty male and 20 proestrous female rats were allotted to four experimental groups with the male groups treated respectively, with olive oil (control), 200, 400, and 800mg/kg body weight of BLCO (p.o) daily for 6 weeks. Each group of untreated female rats (5 rats per group) were mated with each of the four BLCO treated male groups during the last 6^{th} week of BLCO treatment for 7 days according to earlier studies (Raji and Bolarinwa, 1997; Raji et al., 2003; Raji et al., 2006). The presence of a vaginal plug was accepted as the index for positive mating and the day of its appearance was recorded as day 1 of pregnancy. The gestation period of each rat was monitored until delivery. The number and birth weights of offspring, their morbidity and mortality, were recorded (WHO, 1983). Fertility success was calculated using the following formula.

% Fertility Success = Pregnant Females × 100/Mated Females

At the end of the experiments animals of control and BLCO treated groups were sacrificed by cervical dislocation.

Body and reproductive organ weights: The body weights of the animals were recorded, prior to and after treatments and recovery. Testis, epididymis, seminal vesicle, prostate, ovary and uterus were weighed.

Sperm collection and analyses: The epididymis was carefully separated from the testis and the cauda severed from its remaining part. The cauda was quickly transferred to a pre warmed slide (27 °C) and lacerated with a razor. Sperm motility was determined immediately. Sperm was squeezed onto the warm microscope slide and two drops of warm 2.9 % sodium citrate was added. This was covered with a cover slip, examined and scored under the microscope using the ×40 objective of the microscope. Sperm viability (percentage of live spermatozoa) was determined using the Eosin/nigrosin stain. Sperm was squeezed onto a warm microscope slide and two drops of the stain was added. The stained and the unstained sperm cells were counted using ×40 objectives of the microscope and an average for each was taken from which percentage viability was calculated. Sperm morphology was done by staining the sperm smears on warm microscope slides with two drops of warm Wells and Awa stain and air-dried (Wells and Awa, 1970). The slides were examined under the

microscope using ×100 objectives under oil immersion. The abnormal sperm cells were counted and the percentage calculated. Sperm count was done under a microscope with the aid of the improved Neubauer hemocytometer.

***Female fertility study**:* Twenty proestrous female and 20 male rats were randomly allotted to four experimental groups with the female groups treated respectively with olive oil (control), 200, 400, and 800mg/kg body weight of BLCO (p.o) daily for 6weeks. Oestrous cycles of the rats were monitored daily according to the method described by Marcondes et al., (2002). Each group of the BLCO treated female rats (5 rats per group) were mated with each of the four untreated male groups during the last 6^{th} week of BLCO treatment for 7 days according to previous studies (Raji et al., 2005; Raji et al., 2006). The presence of a vaginal plug was accepted as the index for positive mating and the day of its appearance was recorded as day 1 of pregnancy. The date of parturition for each group was noted and recorded. The gestation period of each rat was monitored until delivery. The number and birth weights of the offspring, their morbidity and mortality were recorded (WHO, 1983).

Determination of oestrous cycle: Using Marcondes technique (Marcondes et al., 2002), about 0.1ml of 0.9% saline solution was gently introduced 2-3 times into the vagina of the rat to produce a vaginal lavage. The pipette was withdrawn and its content was placed on a microscope slide and viewed using x40 magnification lens of the microscope. The slides were afterwards fixed in 95% alcohol for 30 minutes and stained using Papanicolaou's staining technique. Oestrous cycle was designated as a four day period extending from the day of proestrus which corresponds with the presence of nucleated vaginal epithelial cells followed by the oestrus phase which is evidenced by the presence of cornified vaginal mucosa cells, metestrus and the diestrus phase, characterized by the presence of leukocytes in the vaginal smear.

Histological study: This was carried out according to the instructions detailed in a previous study (Raji et al., 2003). The testes and epididymes were fixed in 10 % formalin. A thin section (0.05-mm thick) of the tissue was made. The section was stained with hematoxylin-eosin dye. Each slide was clean-blotted and mounted in Canada balsam under a cover slip. A photomicrograph of the slide preparation was taken after examination under the microscope.

Statistical analysis: Data were expressed as mean ± SEM. Statistical significance between the various groups was determined using students *t*-test and ANOVA

RESULTS

Effects of BLCO on body and organ weight: There were no significant differences in body weight of BLCO treated male and female rats when compared with their respective controls. However as shown in table 1, there was a significant dose dependent decrease ($p< 0.05$) in relative weight of the testis and epididymis of BLCO treated rats when compared with the control. There was no significant change in relative weight of seminal vesicles and prostate gland in the BLCO treated rats. Compared with the control, there was no significant change in relative weight of the ovaries and uterus in all BLCO treated female rats (Table 2).

Male fertility study:

Sperm characteristics: As shown in Fig.1 A&B, oral administration of BLCO for six weeks significantly decreased ($p <0.05$) sperm count, sperm motility and viability in a dose-dependent manner, when compared with the control. There was also a dose-related significant increase ($p<0.05$) in the number of sperm with abnormal morphology (Fig. 1C). The most common sperm abnormalities were the bent tail, the curved tail, the bent mid piece and the curved mid piece.

Male fertility success and gestational period: There was 100% mating success as all the female rats cohabited with the BLCO treated and control rats were pregnant within one week of cohabitation. However as shown in Fig. 2A, there was a dose-dependent decrease in gestation period of the BLCO treated rats when compared with the control. There was also a significant decrease ($p<0.05$) in number and birth weight of the offspring from female rats mated with the BLCO treated rats and the BLCO treated females mated with the untreated male rats (Fig. 2B&C). The percentage offspring survival from the rats administered with doses of BLCO showed a statistically significant ($p<0.05$) decrease when

Table 1: Body weight change and relative organ weight of male rats after six weeks of treatment with BLCO

Doses of BLCO (mg/kg b.w)	% Body weight change	Testis(g)	Epididymis (g)	Seminal Vesicle	Prostate (g)
0 Control)	23.20	0.445 ± 0.09	0.081 ± 0.01	0.214 ± 0.01	0.0617 ± 0.02
200	21.60	0.345 ± 0.04*	0.059 ± 0.01*	0.217 ± 0.01	0.0625 ± 0.02
400	15.20	0.354 ± 0.02*	0.059 ± 0.01*	0.226 ± 0.01	0.0625 ± 0.01
800	15.20	0.351 ± 0.03*	0.059 ± 0.01*	0.212 ± 0.01	0.0625 ± 0.01

*P<0.05

Table 2: Body weight change and relative organ weight of female rats after six weeks of treatment with BLCO

Doses of BLCO (mg/kg b.w)	% Weight Change	Relative weight of ovary	Relative weight of uterus
0 (Control)	31.00	0.16 ± 0.003	0.258±0.085
200	26.20	0.12± 0.067	0.244±0.032
400	23.10	0.17± 0.003	0.223±0.055
800	20.20	0.19±0.0025	0.225±0.043

Table 3:

Doses of BLCO (mg/kg b.w)	Oestrous cycle length before BLCO ingestion	Oestrous cycle length after BLCO ingestion
0 (Control)	5.01±0.19	5.02±0.23
200	5.05±0.30	5.09±0.31
400	5.17±0.14	5.02±0.13
800	5.21±0.13	4.76±0.34

Table 4: Frequency of oestrous phases in before and after treatment of female rats with BLCO

Phases of oestrous cycle	Frequency of Oestrous Cycle Phases/Doses of BLCO (mg/kg b.w)			
	0 (Control)	200	400	800
Proestrous- Before	21.73±1.45	21.91±1.92	16.39±2.360	18.22±1.31
Proestrous-After	21.20±2.22	19.82±1.8	17.13±1.64	21.00±1.21
Oestrous-Before	22.25±2.02	22.37±1.84	22.75±2.75	26.11±2.078
Oestrous-After	22.924±2.74	29.59±2.96	27.88±1.63*	30.33±2.03*
Metoestrous-Before	20.42±2.61	20.18±3.37	20.33±1.93	16.11±2.557
Metoestrous-After	21.33±1.46	29.31±3.092	26.63±2.42*	24.89±2.18*
Dioestrous-Before	37.58±2.61	37.44±4.144	40.19±17.51	39.56±3.198
Diestous-After	37.69±3.90	37.69±4.765*	28.25±3.599*	23.78±3.32*

*P< 0.05

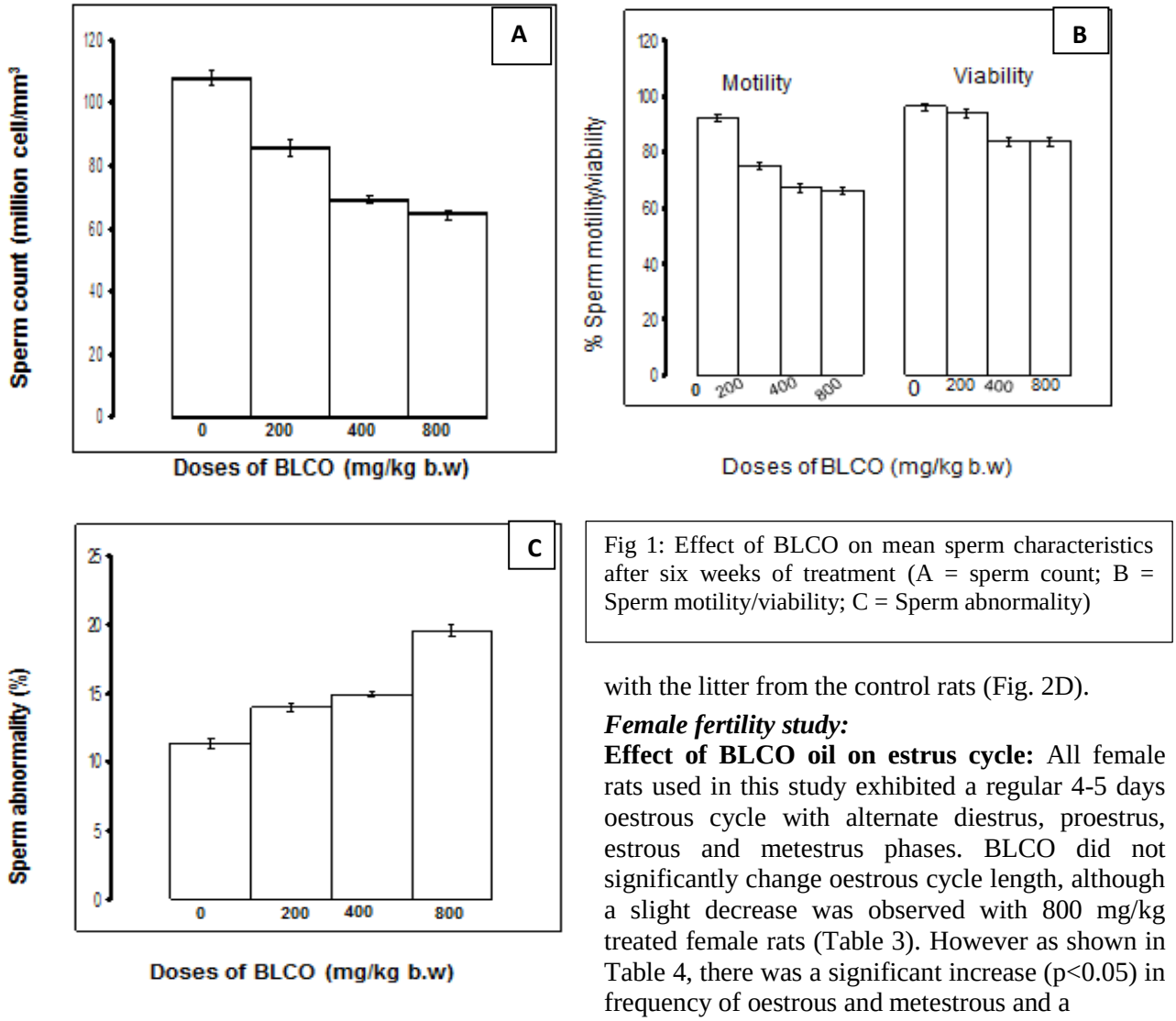

Fig 1: Effect of BLCO on mean sperm characteristics after six weeks of treatment (A = sperm count; B = Sperm motility/viability; C = Sperm abnormality)

with the litter from the control rats (Fig. 2D).

Female fertility study:

Effect of BLCO oil on estrus cycle: All female rats used in this study exhibited a regular 4-5 days oestrous cycle with alternate diestrus, proestrus, estrous and metestrus phases. BLCO did not significantly change oestrous cycle length, although a slight decrease was observed with 800 mg/kg treated female rats (Table 3). However as shown in Table 4, there was a significant increase ($p<0.05$) in frequency of oestrous and metestrous and a

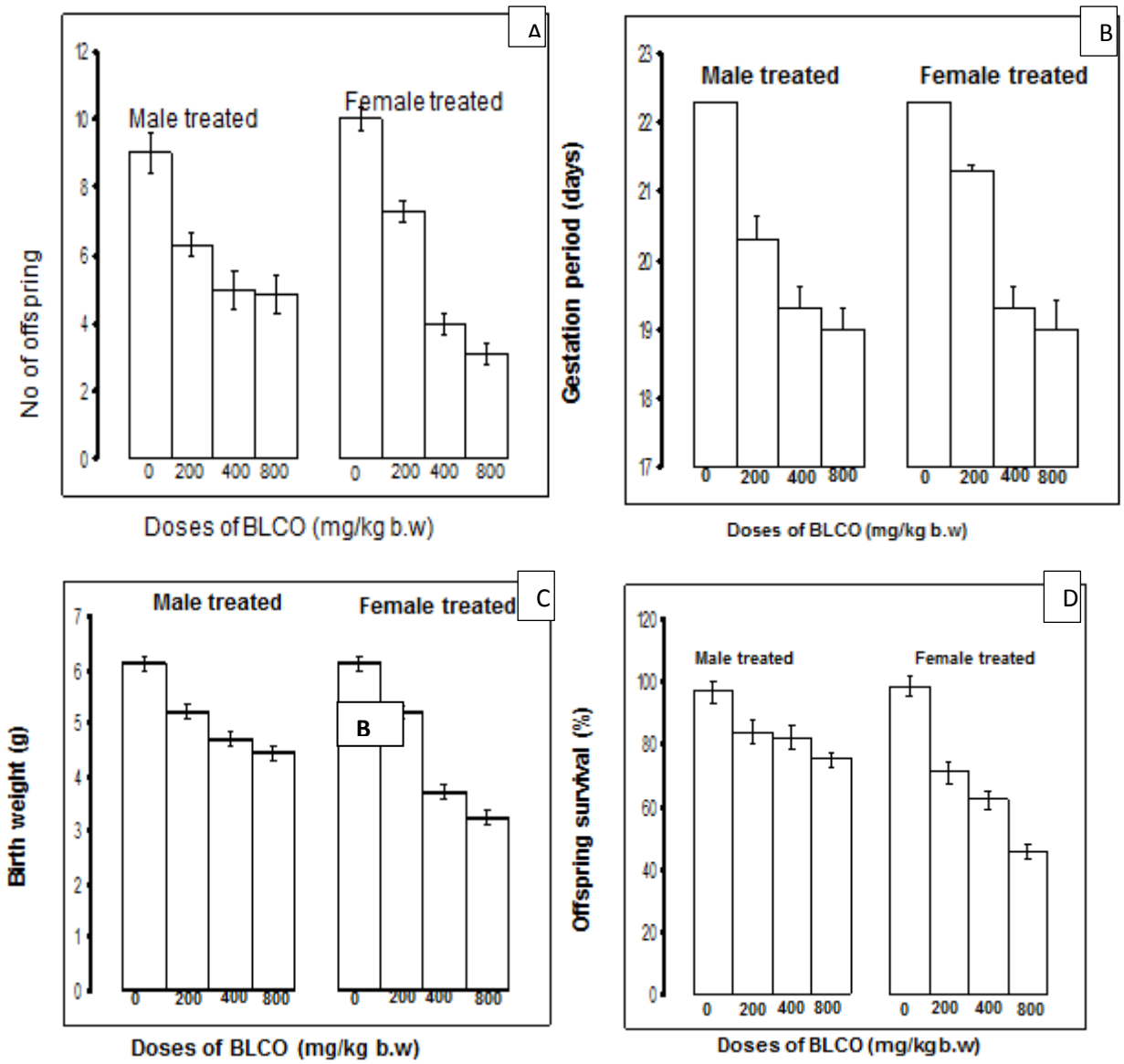

Fig. 2: Effect of BLCO on fertility indices of male and female BLCO treated rats [A = Gestation period by untreated female mated with treated male (Male treated) and untreated males mated with treated females (Female treated); B = Number of offspring delivered; C = Birth weight of offspring and D = Percentage offspring survival after 24 hours of normal delivery]

Table 5: Mating success in female rats treated with BLCO for 6 weeks

Doses of BLCO (mg/kg b.w)	Number of mated female rats (n = 5)	% Mating success
0 (Control)	5	100
200	4	80
400	3	60
800	3	60

BLCO treatment. These changes were BLCO dose-dependent.

Female fertility success and gestational period: There was a dose-dependent decrease in the number of BLCO treated female rats that were pregnant when compared with the control (Table 5). Similarly there was also a dose-dependent decrease in gestation period exhibited by these rats when compared with the control (Fig. 2A, Female treated). The number and birth weight of the offspring also significantly decreased ($p<0.05$) in BLCO treated rats (Fig. 2B&C). The percentage litter survival from BLCO treated rats also showed a statistically significant ($p<0.05$) decrease when compared with the control (Fig. 2D).

Histology of the testis and caudal epididymis: Fig. 3 shows representative photomicrographs of control and BLCO-treated epididymes. As shown in the figure, BLCO produced dose-dependent lesions in the epididymis ranging from mild to severe epithelial degeneration and significant decrease ($p< 0.05$) in the tubular contents. Similarly, treatment-related lesions were observed in the testes ranging from severe congestion of interstitial tissue, oedema with few vacuolization and decreased germinal epithelium with increased number of vacuolization (Fig. 4).

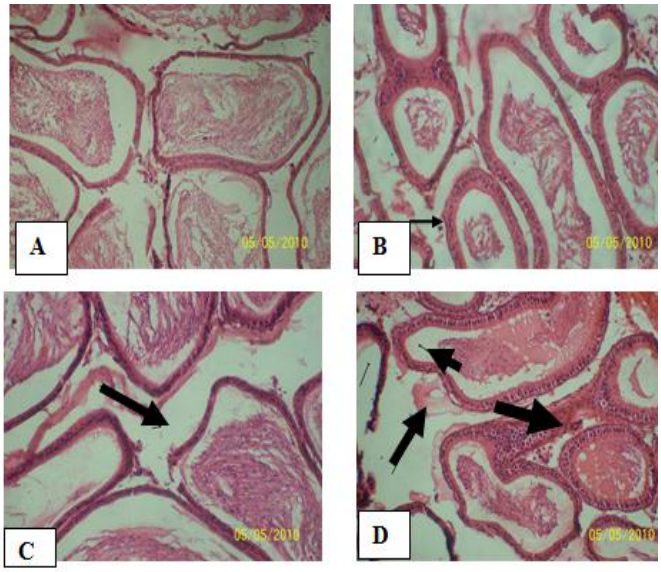

Fig. 3: Photomicrograph of caudal epididymis (A control, olive oil treated) showing no visible lesions, B; 200mg/kg b.w, C; 400mg/kg b.w, D; 800mg/kg b.w BLCO treated rats. Mild to moderately severe interstitial oedema (arrows) (mag. X40)

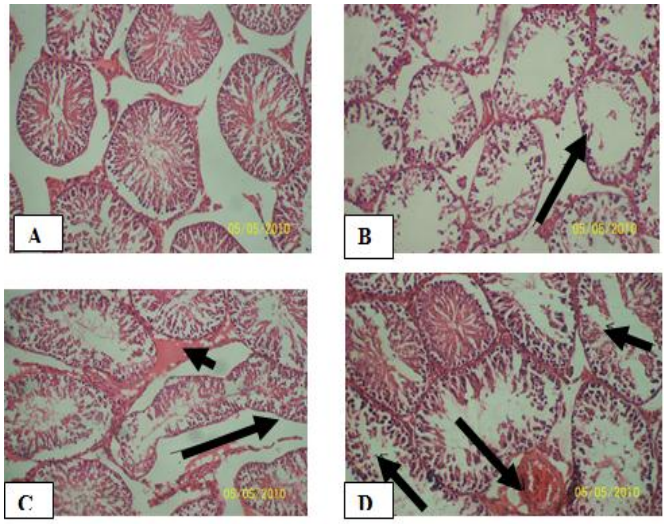

Fig. 4: Photomicrograph of testes of low dose BLCO treated rats, (A; Control olive oil treated) showing no visible lesions, B; 200mg/kg b.w showing mildly severe interstitial oedema (arrow) C; 400mg/kg b.w showing mild interstitial oedema germinal erosions (arrows) and D; 800mg/kg b.w showing interstitial oedema and marked interstitial congestion, with slight reduction in germinal layer (arrows) (mag. X40)

DISCUSSION

Accumulating scientific evidence has shown that many man-made and naturally occurring substances released frequently into the environment have adverse effects on the endocrine system of humans and wildlife (Cooper et al., 1997). Some of these endocrine-disrupting chemicals (EDCs) mimic natural estrogen, whereas others may function as anti-estrogen (Karel et al., 2003; Aerni et al., 2004). A number of environmental chemicals have been identified as endocrine disruptors, and their unwanted effects on lives have become serious problems all over the world. The adverse effects on endocrine systems in animals have resulted in reproductive malfunction and developmental disorders (Indarto and Izawa, 2001).

Although, various studies have been carried out on crude oil, very few have been directed at its impact on reproductive system. Increasing concern about the possible declining trend in fertility of man and wildlife animals over the past decades as a result of exposure to various environmental pollutants such as estrogenic agents and aromatic hydrocarbons have been widely reported (Lyons et al., 1999; Onwurah, 2002; Izegbu et al., 2005; Shittu et al., 2007; Shittu et al., 2008). Previous studies focused on the male showed that BLCO significantly reduced sperm count, sperm motility and normal morphology within seven days of administration (Oriskwe et al., 2004; Adesanya et al., 2009; Farombi et al., 2010). Whether or not the seven day administration of BLCO would affect fertility indices of these rats was not reported in these studies considering the fact that spermatogenic cycle of rats is about 10-14 days as reported in several studies (Leblond and Clermont, 1952; Clermont 1972; Johnson et al., 1980; Sinha et al., 1985; Laura et al., 1993; Jones, 1999; Breed and Taylor, 2000; Peirce and Breed, 2001).

We carried out the present study to investigate the effects of Bonny Light Crude Oil ingestion on fertility indices of male and female albino rats during long term exposure to target several cycles of spermatogenesis. The effects of BLCO on female reproduction/fertility are also reported for the first time. BLCO was dissolved in olive oil. It should be noted olive oil used to dissolve BLCO did not produce any adverse effect on the measured parameters. Previous study (Owu, et al; 2005) has shown that olive oil improved palatability and taste of BLCO for consumption by the rats, and also serves as an antioxidant which reduces the gastrointestinal side effects of BCLO. It is worthy to note that crude oil is used in combination with olive oil in folklore medicine in some parts of the Niger Delta region of Nigeria (Dede, et al., 2002). Other beneficial health effects of olive oil have been documented (Keys et al., 1986; Alarcon et al., 2001). The findings from the present study showed that BLCO ingestion over a period of time could pose serious threat to fertility as it significantly reduced fertility indices in both male and female rats. The weight of the reproductive organs of the treated rats showed variable responses to the BLCO administration. The relative weight of the testis and caudal epididymis showed a dose

dependent decrease which correlates with the histopathological treatment-related lesions of these organs that were characterized by severe congestion of interstitial vessels, decreased germinal epithelium, and increased number of vacuolization. The epididymis acts as a storage organ for mature spermatozoa. The decrease in weight of epididymis observed in the BLCO administered rat groups was accompanied by significant dose dependent decrease in sperm count and fertility.

The reduction in sperm viability (percentage live sperms) of BLCO treated rats corroborates the previous reports (Oriskwe et al., 2004; Farombi et al., 2010) albeit during short term exposure of rats to BLCO. This correlates with the various degrees of histological lesions of the testes including interstitial oedema which could have been compromised and might result in decrease testosterone level as previously reported (Otitoju et al., 2007). Georgewill and Nwankwoala, (2006) also reported that prolonged exposure of guinea pigs to Nigerian crude oil decreased the level of their reproductive hormones. The morphological abnormalities observed in the sperm cells of the BLCO treated rats was in the form of tailless head, headless tail, curved tail, bent tail, bent mid piece, rudimentary tail, curved mid piece, looped tail, and twin head. The most common abnormalities observed were the bent tail, curved tail, bent mid piece, and the curved mid piece. These secondary abnormalities usually occur during epididymal transport, maturation and storage of sperm, during which period the spermatozoa develop motility. The observed significant decrease in the average number and birth weight of offspring delivered by the untreated female rats that were mated with BLCO treated male rats, could be caused by the significant reduction in the epididymal sperm motility seen in the BLCO treated rats. Low sperm count and motility and the high percentage of abnormal spermatozoa level have been associated with reduced fertility in many animal species including rats (Gadea, 2005; Kastelic and Thundathil, 2008; Love, 2011)

The variation observed in the gestational period could probably be responsible for the decreased birth weight and low survival ratio of the offspring produced by the untreated female rats mated with BLCO treated male rats. The health problems associated with oil spill may be through any or combinations of several routes, including, contaminated food and / or water, emission and / or vapors. Toxic components in oil have been shown to exert their effects on man through inhibition of protein synthesis, nerve synapse function, and disruption in membrane transport system and damage to plasma membrane (Prescott et al., 1996). Crude oil hydrocarbons can affect genetic integrity of many organisms, resulting in carcinogenesis, mutagenesis, low birth weight, impairment of reproductive capacity and possibly death (Short and Heintz, 1997).

Our study demonstrated that BLCO alters the estrous cycle, by prolonging the duration of the oestrous and metestrous phases and subsequently lowering the frequency at which the diestrous phase occurs. Consequently the frequency of ovulation was reduced and fertility might therefore be impaired. The effect of BLCO administration on the estrus cycle length was insignificant as there was no marked difference in the pre-treatment and post-treatment estrus cycle length when compared within the group. However, at each phase of the cycle there were varying differences between some of the pre-treatment phase and post-treatment phases. This indicates a disruption in oestrous cycle activity which could lead to disruption in ovarian cycle activities. An increase in estrous phase and metestrus phase indicates the availability of matured Graafian follicles and the occurrence of ovulation (Shrestha et al., 2010) which in the present study was most probably pseudo-ovulation with the consequent loss of fertility. In rats, estrogen peaks on the day of proestrus. There is usually a surge of progesterone, LH and FSH just before ovulation (McCracken et al., 1999). At metestrous phase, corpus luteum is formed and the uterus lining secretes progesterone, at the diestrus phase, the corpus luteum now actively secretes progesterone. The period of increased progesterone secretion which is known as the luteal phase in human is known as the metestrus phase in rats. At diestrus, estradiol levels are low (Janet et al., 2003). The shorter duration of the post-treatment diestrus phase further aggravated disturbed ovarian cycle, reduction in oocyte number and finally reduced fertility and reduction in number of viable offspring.

The result of this study suggests that Bonny light crude oil has a negative effect on fertility of male and female rats. It is not known whether the reproductive impairment caused by BLCO in male and female rats is reversible. The involvement of reproductive hormones in this anomaly is also not known at present. Ongoing studies in our laboratory are expected to address these and other issues.

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