ASSESSMENT OF THE LITTER SIZE, BIRTH WEIGHT AND SERUM HORMONAL PROFILE OF RATS ORALLY EXPOSED TO CRUDE OIL.

PRESENTED BY

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INTRODUCTION:

- Oil exploration and exploitation has brought about myriads of environmental problems arising from oil spillage and gas flaring. Large volumes of crude oil are spilt annually into the creeks, resulting in soil and water pollution (Georgewill, 2009). The adverse effect of oil spillage on the inhabitants of this region may be attributed to consumption of oil polluted water, contaminated agricultural produce and sea foods.

- Several studies have shown the impact of crude oil on various laboratory animals and by extension humans. The effect of crude oil on the biochemical, haematological and spermatogenic properties of laboratory animals have been extensively elucidated (Eyong, 2002; Dede *et al.*, 2005; Otitoju and Onwurah, 2006; Fischer *et al.*, 2009). Various studies have shown a high degree of toxicity of crude oil, diesel and kerosene, however there is need to assess how exposure to extracts of crude oil affect the reproductive functions of the parents and the health status of the offspring (Uboh et al. 2008; Otitoju, O. & Onwurah, I. N. E. 2007).

- A number of chemicals with the capacity to disrupt the endocrine and reproductive systems are now widespread in the environment Colbborn *et al.*, (1993). These consist of synthetic and naturally occurring chemicals that affect the balance of normal hormonal functions in animals including humans **(**McClellan-green, 2003**)**.

Introduction Cont'd

- Exposure to crude oil has been reported to cause different toxicity effects, including reproductive toxicity in experimental rats (Otitoju *et al.*, 2006; Oveh and Nwankwoala 2009).
- This study assessed the serum sex hormonal profile of male and female rats orally exposed to crude oil, and the litter size, birth weight and tail length of their first generation offsprings

MATERIALS AND METHODS:

- **Animal Handling and Treatment:** Thirty six apparently normal matured albino Wistar rats (12 males and 24 females weighing180 to 200g), obtained from Biochemistry Department Experimental Research Animal House of the University of Calabar, Calabar, Nigeria, were used in this study.

MATERIALS AND METHODS:

- The animals were distributed into two major groups as shown in Table 1
- They were fed with standard laboratory diet and tap water, and kept under 12 hours light/dark cycle illumination and room temperature.
- Crude oil (60mg/kg body weight) was administered to rats in group 2 for 28 days, based on LD_{50} and reference to literature, after which they were mated at the ratio of 1 male : 2 females.

Materials and Methods Cont' d

- Administration continued until the female rats were confirmed pregnant, and lasted till delivery.
- On delivery, the litter size, birth weights and tail length of the litters were measured.

Materials and Methods Cont'd

- The offspring were sacrificed, after 6 weeks of birth and blood samples collected for analysis.
- The second set of male and female rats were sacrifice after 28 days of exposure to crude oil.
- The study was carried out according to the Guidelines of the Institution's Animal Research Ethics Committee, with reference to the Guide for the Care and Use of Laboratory Animals (NRC, 1995).

- **Collection and preparation of blood for analyses:** Blood samples were obtained from rats by cardiac puncture, under chloroform vapor anaesthesia, into sterile plain sample bottles for sex hormonal assay.

Materials and Methods Cont' d

- **Hormonal Assay:** FSH, LH, Progesterone, Oestrogen and Testosterone were analysis using enzyme linked immunosorbent assay (ELISA). Analytical grade reagent kits were used in this analysis. Basically, enzyme immunoassays combined the specificity of antibodies with the sensitivity of simple spectrophotometric enzyme assays by using antibodies or antigen coupled to an easily assayed enzyme which possessed a high turnover number.

- The optical densities were read using a micro plate reader at specific wavelengths within 30 minutes.
- **Measurement of Body weight and Tail length:** Measurements were done using electronic weighing balance and a simple metric measuring tape respectively.
- **Statistical Analysis:** Results were presented as mean ± S.E.M and statistically analysed using one-way analysis of variance (ANOVA) with SPSS window statistical software programme. Student "t" test was used for pair-wise comparison, and differences were considered significant at $p<0.05$.

TABLE 1

Distribution and treatment of animals in the respective equipment groups

Group	Treatment	Number of Rats	
		Males	Females
1	Distilled Water (control)	6	12
2	60mg/kg body weight of crude oil	6	12

RESULTS:

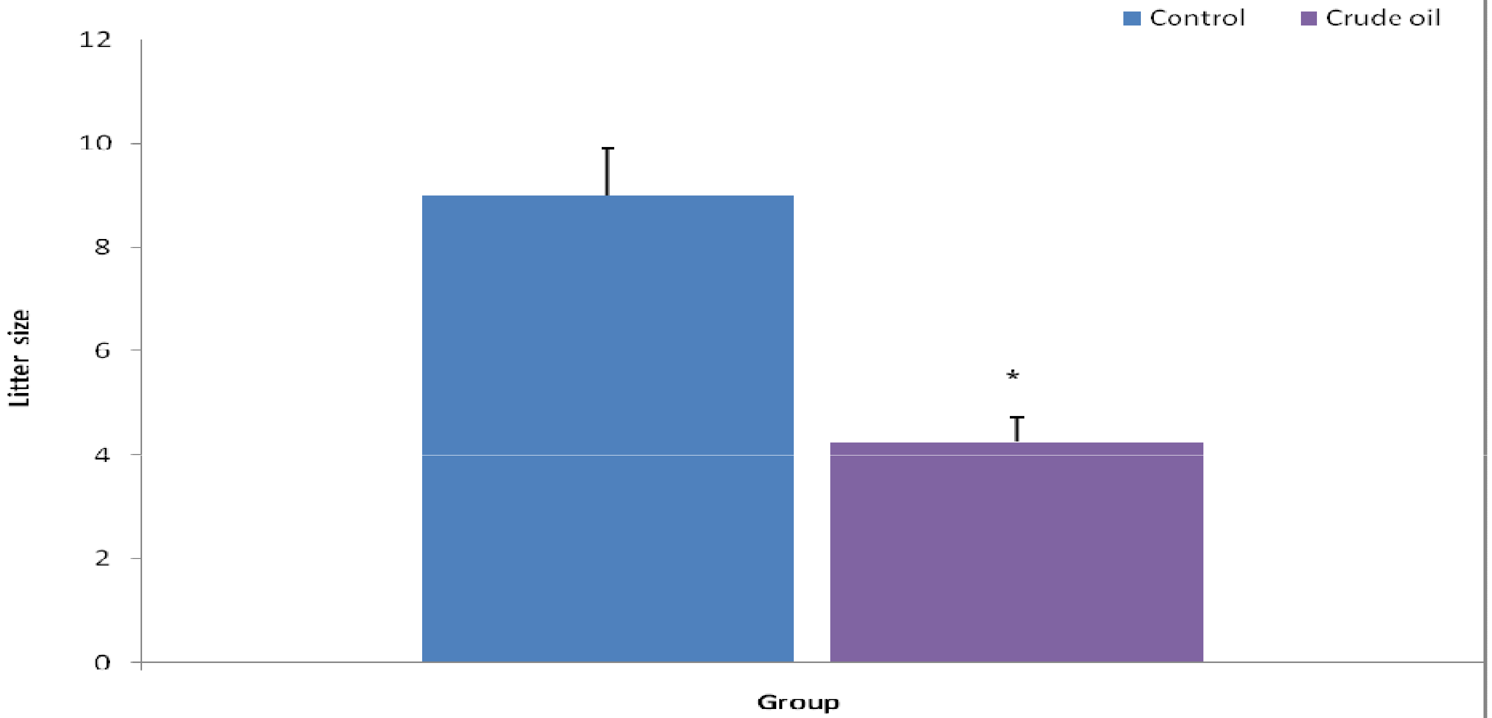

Figure 1: Comparison of the litter size from experimental rats.
Values are expressed as mean ± SEM
***significantly different from control at p<0.05;**

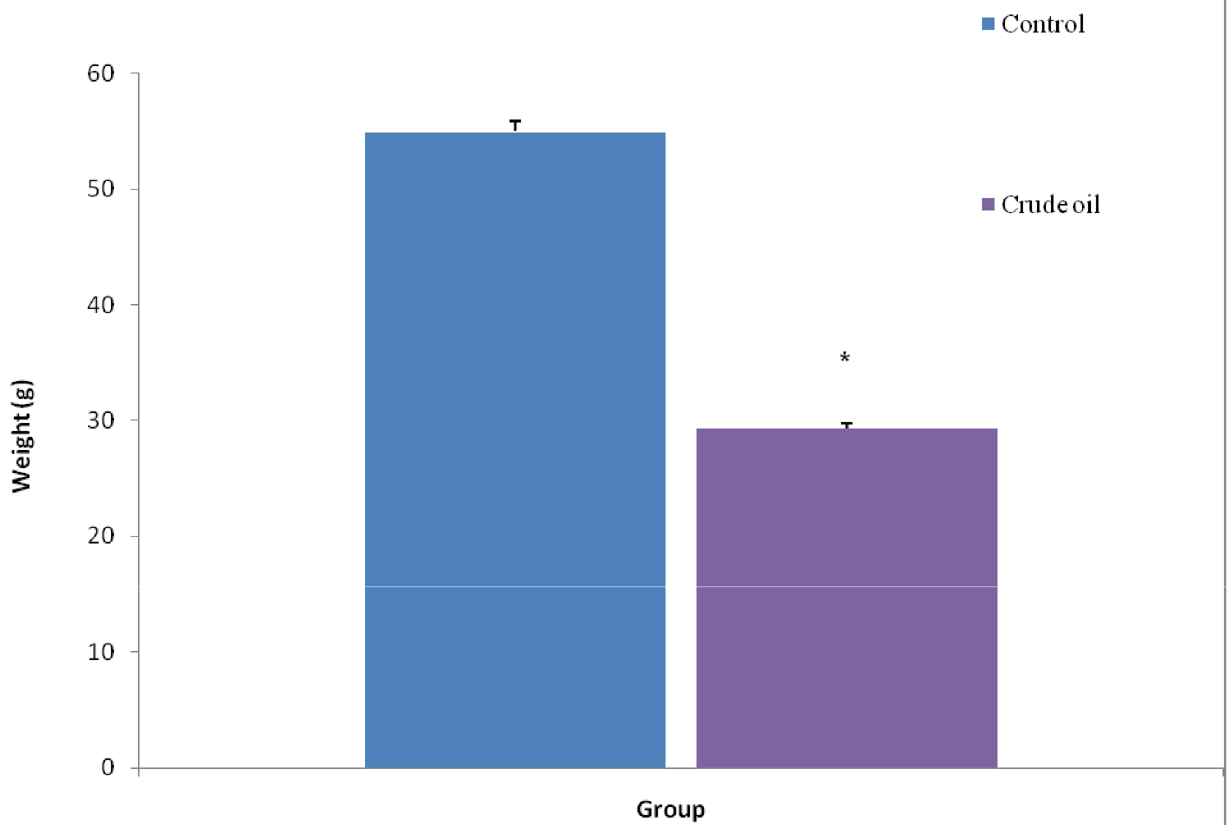

Figure 2: Comparison of litter weight, at delivery, from experimental rats.
Values are expressed as mean ± SEM
***significantly different from control at $p<0.05$;**

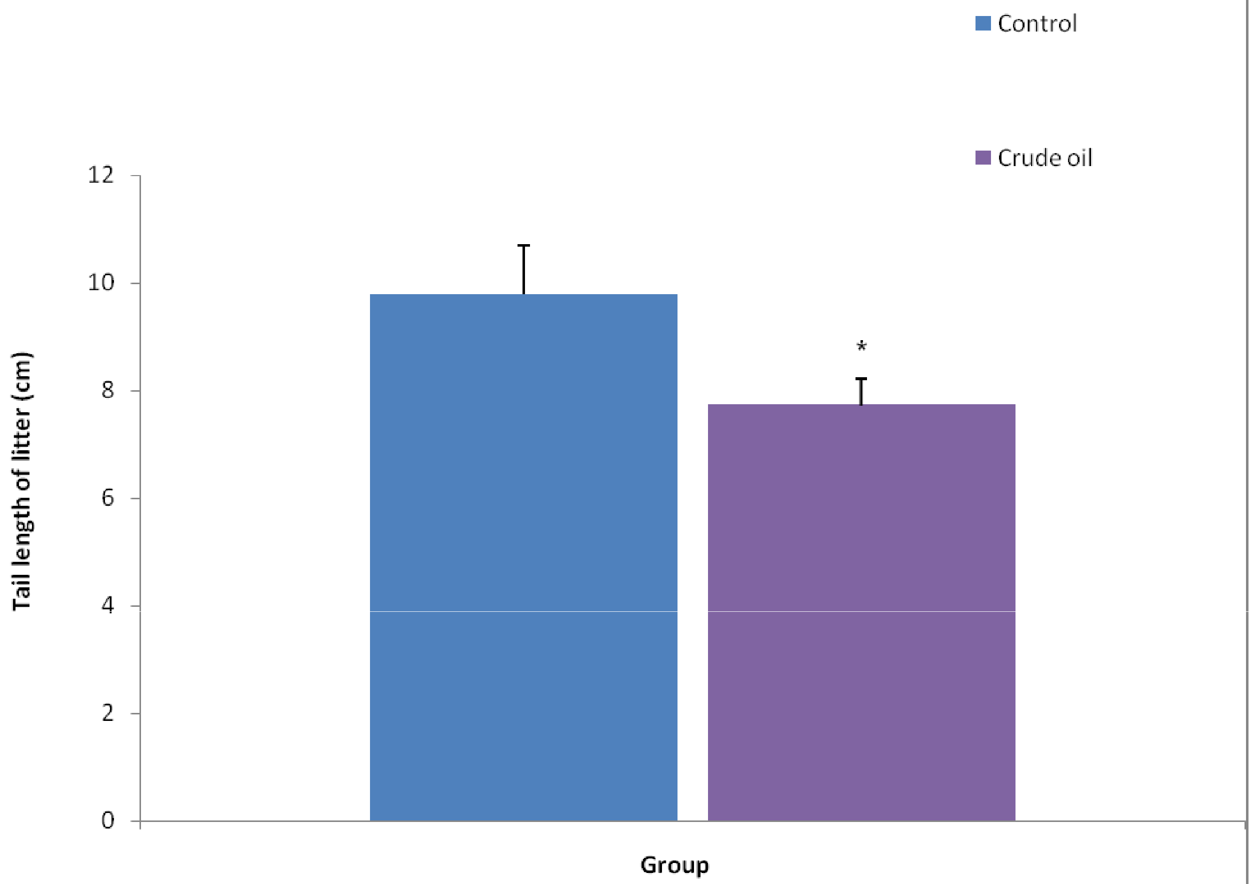

Figure 3: Average litter tail length between different experimentalgroups of rats, after six weeks.
Values are expressed as mean ± SEM, n = 7.
***significantly different from control at p<0.05;**

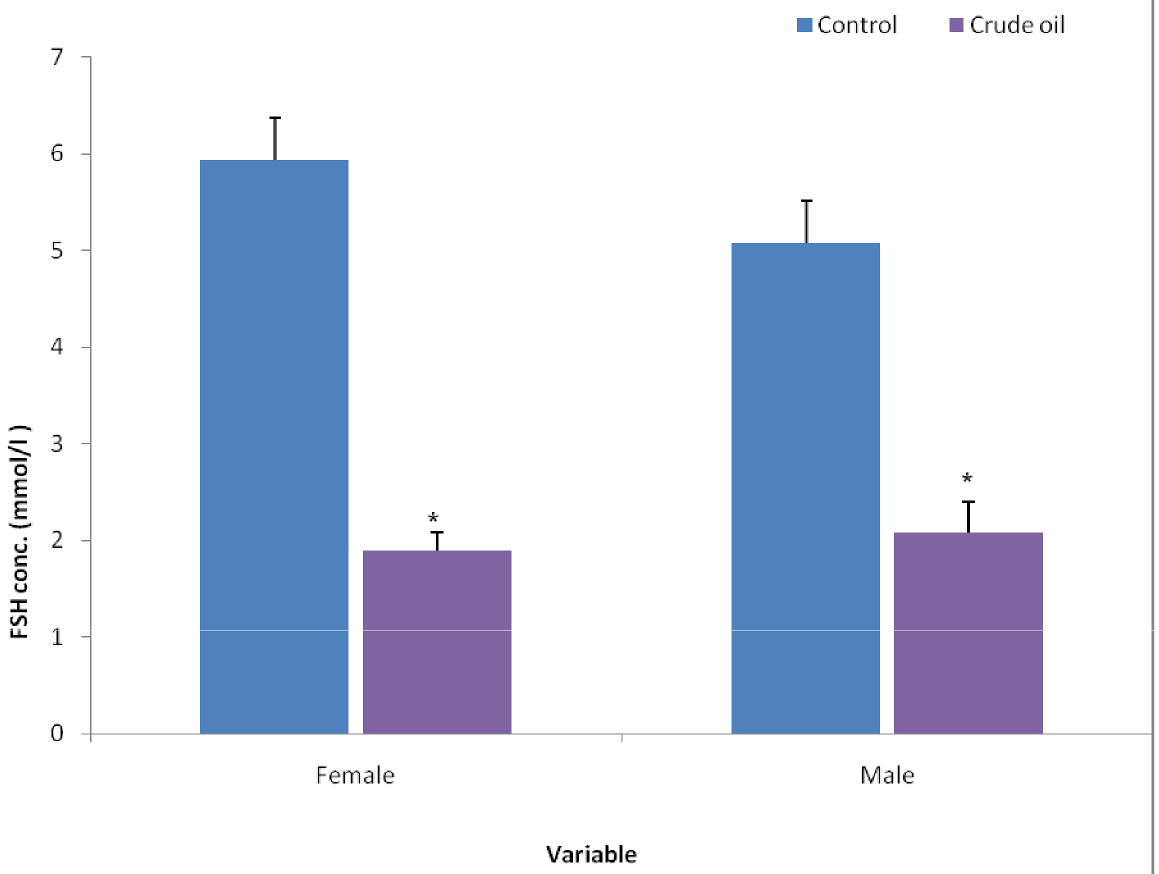

Figure 4: Comparison of FSH concentrations between different experimental groups of male and female rats.
Values are expressed as mean ± SEM, n = 6
***significantly different from control at $p<0.05$;**

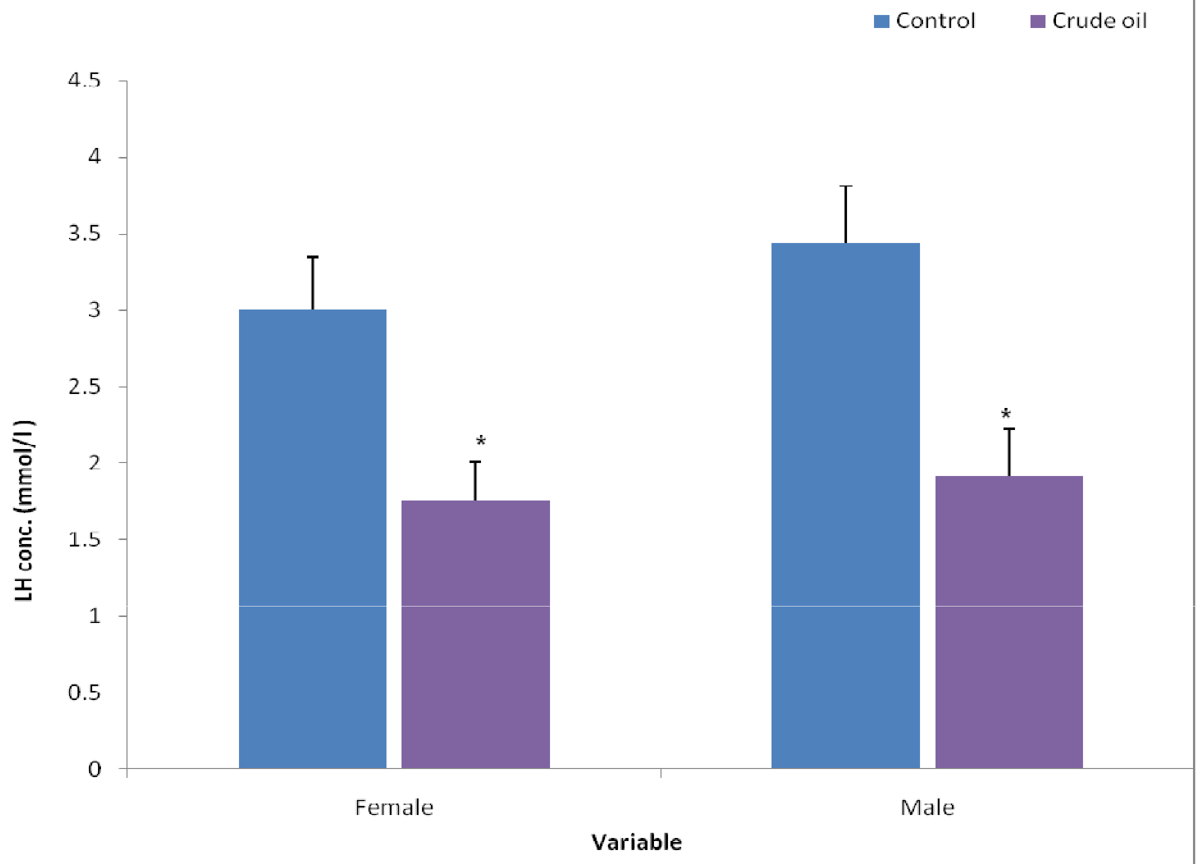

Figure 5: Comparison of LH concentrations between different experimental groups of male and female rats.
Values are expressed as mean ± SEM, n = 4
***significantly different from control at $p<0.05$;**

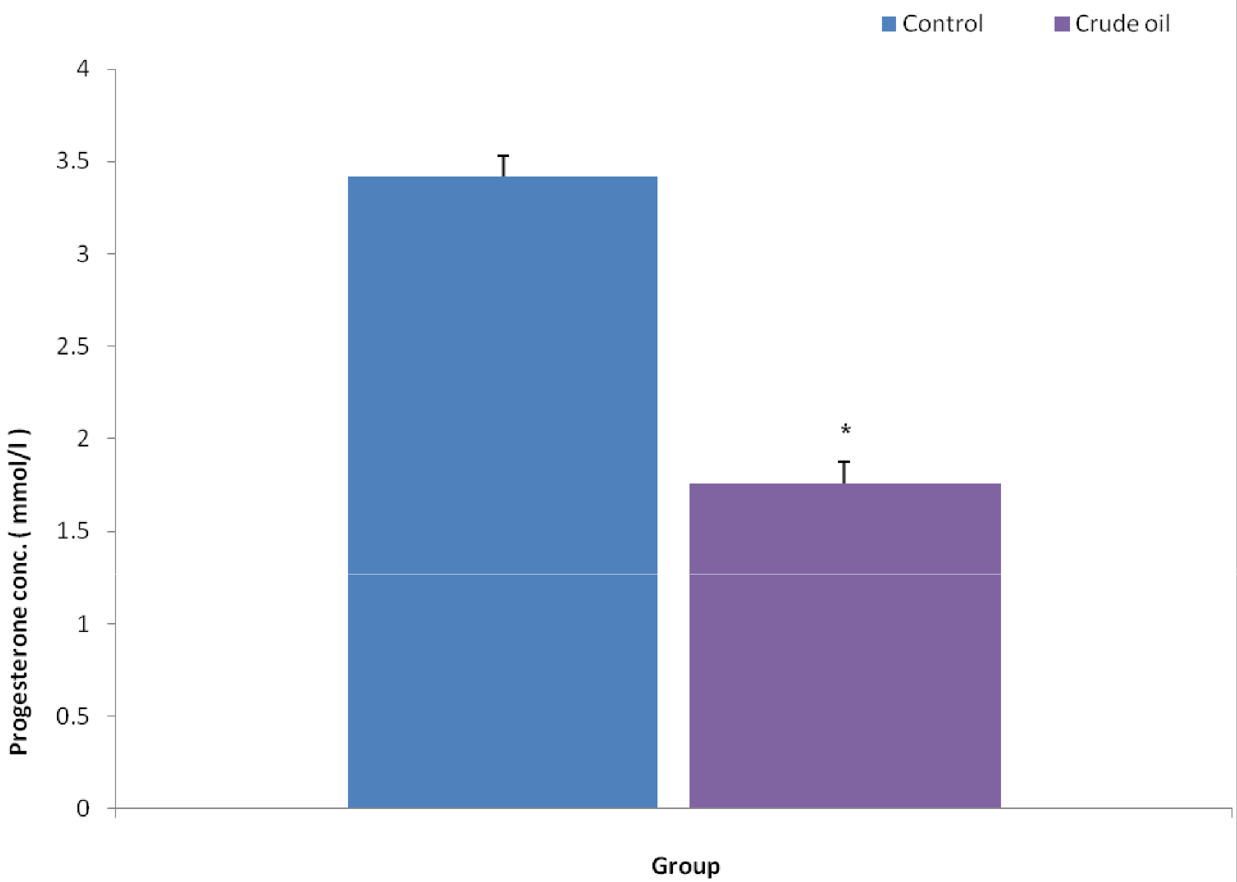

Figure 6: Comparison of progesterone concentrations between different experimental groups of female rats.
Values are expressed as mean ± SEM, n = 6
*significantly different from control at $p<0.05$;

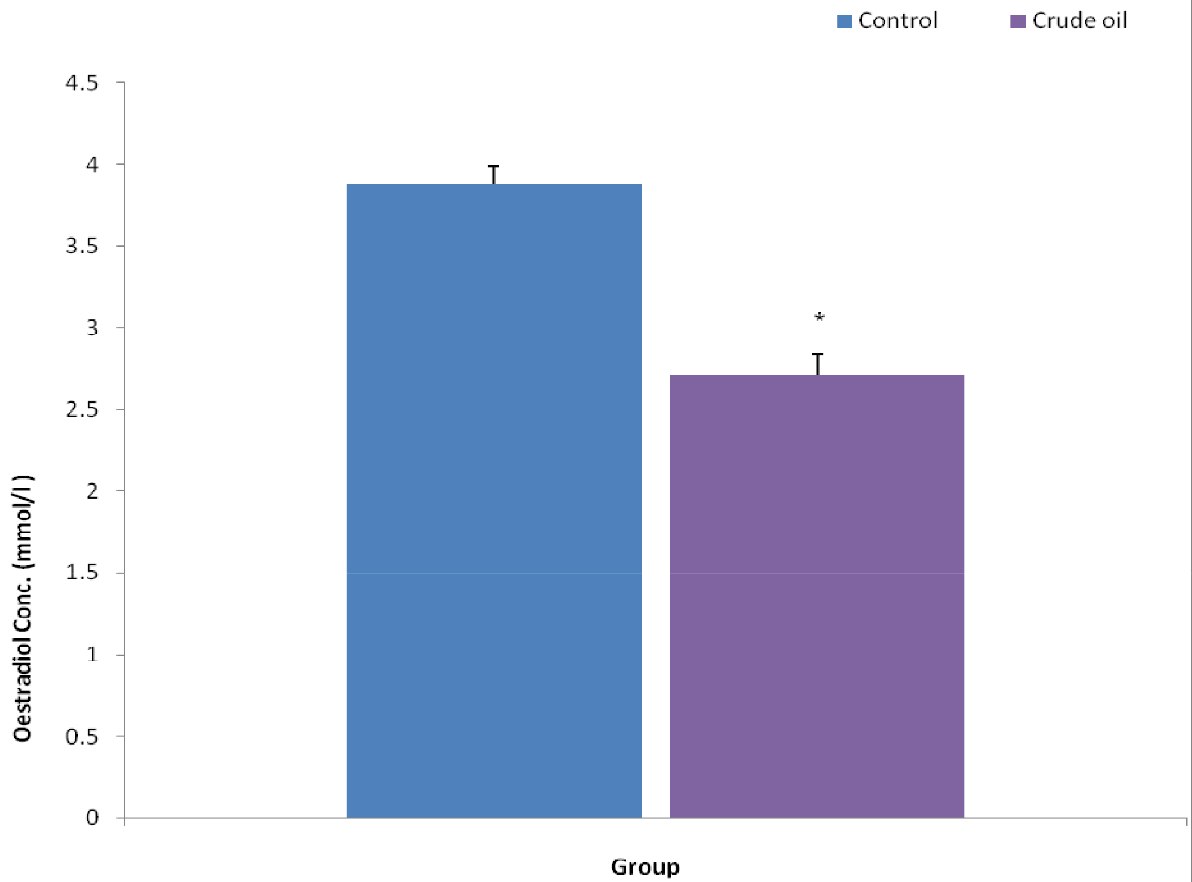

Figure 7: Comparison of Oestradiol concentrations between different experimental groups of female rats.
Values are expressed as mean ± SEM, n = 6
***significantly different from control at p<0.05;**

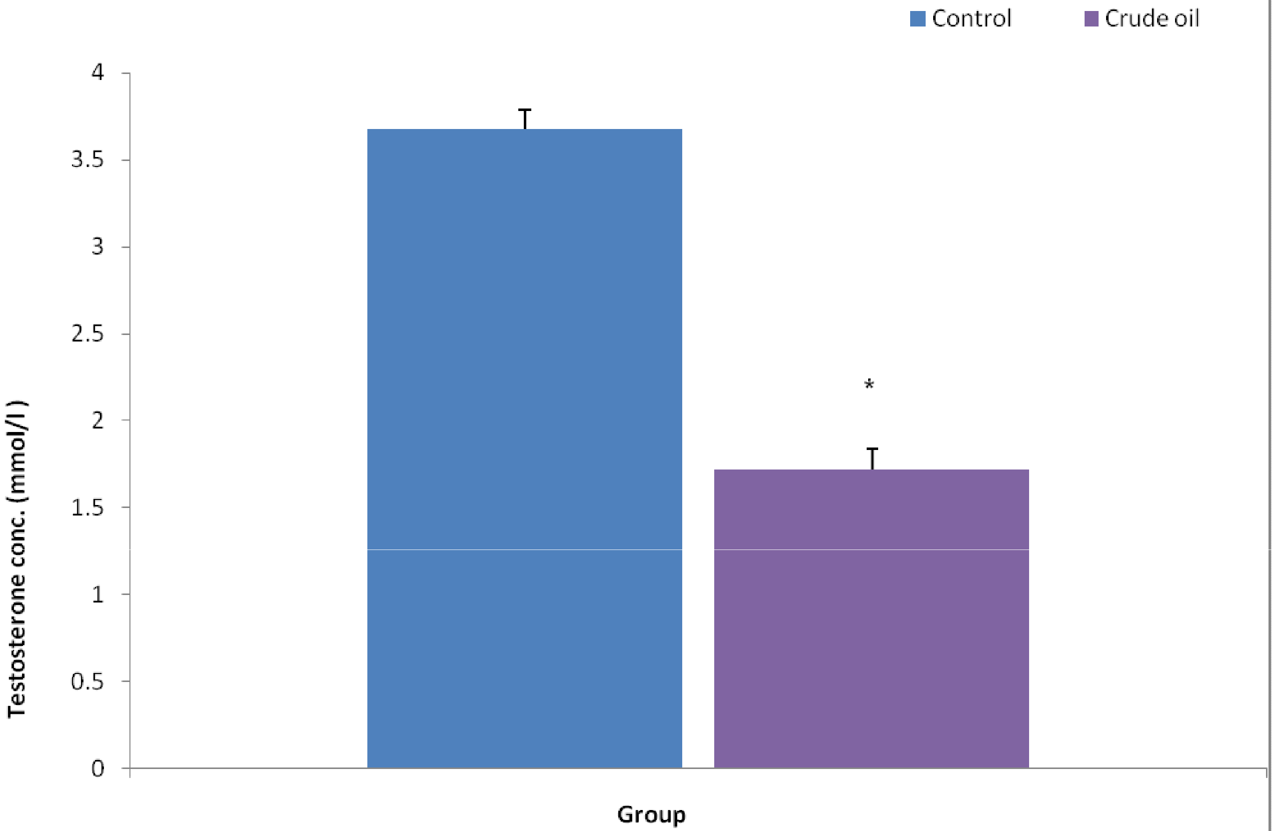

Figure 8: Comparison of testosterone concentrations between different experimental groups of male rats.
Values are expressed as mean ± SEM, n = 6
***significantly different from control at $p<0.05$;**

RESULT & DISCUSION:

- The use of such biological markers as sex hormonal profiles (including FSH, LH, progesterone, oetradiol and testosterone) and fecundity to assess the effect of chemical pollutants on the reproductive functions has been reported in both animal and human subjects (Evanthia, D. K et al. 2009).
- Based on these reports, the plasma sex hormonal concentrations may be used to monitor the functional integrity of the reproductive tissues and the toxicity effects of some environmental toxicants.

Result & Discussion contd.

- The results showed that exposure to crude oil produced low fecundity (as shown by significant reduction in litter size), a significant decrease in FSH, LH, progesterone and oestradiol levels in females, and decrease in FSH, LH and testosterone levels in male rats.
- Also, low birth weights were observed in this study to be associated with exposure to crude oil. These results agree with the results of the study reported by Uboh *et al.* (2012) for rats exposed to gasoline vapours. According to the authors, exposure to gasoline vapours significantly reduced sex hormonal levels in rats.

Result & Discussion contd.

- The low fecundity and reduction in sex hormonal levels reported in this study gave an indication of crude oil induced endocrine disruption which might have pre disposed the animals to reproductive dysfunctions. Reduced birth weights and premature birth have been reported to be associated with exposure to cadmium during human pregnancy (Henson and Chedrese, 2004).

Result & Discussion contd.

- Also, reduced fertility and low birth weights, indications of reproductive toxicity, have been reported in humans occupationally exposed to such heavy metals as Cd, Pd and Hg; and petrochemical solvents (Andrews *et al.*, 1994; Anttila and Sallmen, 1994; Lindbohm, 1995; Gerhard *et al.*, 1998; Grazuleviciene *et al.*, 1998; Herz-Picciotto, 2000).

Result & Discussion contd.

- Endocrine disruption is reported to be one of the mechanisms implicated in reproductive toxicity induced fertility disorders (Moran *et al.*, 2003; O'Connor and Chapin, 2003; Hernandez-Ochoa *et al.*, 2009), and the adverse effects of endocrine active substances on reproduction and development have been evaluated (O' Conor and Chapin, 2003).

- The mechanism of action of endocrine disruptors-induced reproductive toxicity may be through their activities as antiandrogens (Kavlock *et al.*, 1996; Crisp *et al.*, 1998), aromatase inhibitors (Hirsch *et al.*, 1986, 1987), and testosterone biosynthesis inhibitors (Bhasin *et al.*, 1986; Heckman *et al.*, 1992).
- Such heavy metals as Cd and Pb, and hydrocarbons as PAHs, polychlorinated dibenzofurans and polychlorinated biphenyls have been reported to be potent endocrine disrupting agents (Peterson *et al.*, 1993; Kelce *et al.*, 1995; Sharpe and Irvine, 2004).

Result & Discussion contd.

- Birth weights and tail lengths (in such experimental animals as rats) have been reported to play an important role in assessing chemical pollutants induced developmental toxicity (Hougaard *et al.*, 2008). In this study, remarkable low birth weights and tail lengths were recorded for litters delivered from the mating of male and female rats exposed to crude oil.

Result & Discussion contd.

- Other authors reported similar observations from rats fed with crude oil contaminated feed (Mitchell *et al.*, 2004). According to the report of these authors, a significantly low body weights were recorded for litters produced by pregnant rats fed on crude oil contaminated feed, and impregnated by male adult rats also fed with the contaminated feed.

Result & Discussion contd

- Relatively shorter tail lengths were recorded for litters produced from the mating of male and female rats exposed to crude oil. Decrease in birth weights and tail lengths are among the physical manifestations of foetal developmental toxicity. Although the specific mechanism(s) of foetal developmental toxicity is (are) not very clear, Schettler (2001) reported that prenatal exposures to both methyl-mercury and polychlorinated biphenyls (PCBs) are related to impaired neurodevelopment in infants.

- The results of this study showed that exposure to crude oil can induce low birth weights and relatively shorter tail lengths in litters delivered by exposed female rats, mated with exposed male rats.

Conclusion:

- Decreased serum sex hormones levels, litter size, birth weight and tail length are reported in this study for the first generation offspring from male and female rats exposed to crude oil.
- It may therefore be concluded from the results of this study that exposure to crude oil induce reproductive and developmental toxicity in rats

THANK

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