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Commonly Used Hydrocarbons (Petrol and Diesel) Adversely Affects Male Rats Reproduction

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Abstract: Every barrel of crude oil holds remarkable potential to keep us warm to keep us on the going and to provide the building blocks for countless products we depend on everyday meanwhile, its environmental hazards have not been giving adequate attention as it affects man, especially in the line of reproduction as one of the characteristics of living thing. Thus, the present study investigates petrol and diesel possible effects on rats' reproduction. About 25 healthy male wistar rats weighed between 93-230 g were randomly divided into 5 groups: A-E, each containing 5 rats. Groups A and B were orally dosed with 0.2 and 0.4 mL/rat of petrol, respectively likewise groups C and D were dosed with 0.2 and 0.4 mL/rat of diesel and the group E served as control and received distilled water. The dosing was done every day for 28 days. No significant ($p>0.05$) change in body weight of the rats. Likewise, the sperm counts of the treated rats showed no statistically significant results when compared both petrol and diesel treated rats with the control. Significantly, abnormal spermatozoa morphology and low percentage of motile spermatozoa was evident in both petrol and diesel treated rats ($p<0.05$). However, histopathology revealed in a dose related manner seminiferous tubules without lumen, distruption of histoarchitecture of seminiferous tubules with vascular congestion of the interstitium in petrol treated tissues while the diesel treated rats at same doses exhibits shrunk, conjoin and elongation of seminiferous tubules and lost of sertoli cell. These findings indicate antifertility properties of the two hydrocarbons.

Key words: Petrol, diesel, testicular histology, sperm parameters, reproduction, rat

INTRODUCTION

Crude oil is widely used because of its combustibility, density and efficiency. Crude oil is a complex mixture of many different components (oil and an assortment of chemicals that include acids, alkalis, phenols, sulphides, hydrocarbons, heavy metals, mercaptan and other toxic components).

All crude oil are essentially hydrocarbons, the difference in properties especially, the variation in molecular structure. In the reservoir it is usually found in association with natural gas (Thomas, 2000). Crude oil is basically heated to very high temperature until the hydrocarbons that make up the crude oil separate into jet fuels, gasoline, kerosene, light virgin naphtha, petroleum spirit, petroleum naphtha, diesel fuel oil (Asbury, 1942; Chris, 2007). Exposure to crude oil may occur during drilling, transporting and refining. Accidental spill-age accounts for a more serious exposure to crude oil by wildlife and humans. An example of such spillage occurred in Nigeria in November, 2002 (WWF, 2002).

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 at one time or the other. 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.

However, majority of the people in the communities ingest crude oil either directly as curative agents for antipoisoning (snake venom antidotes), anti-convulsion, treatment of skin infection e.g., scabies or indirectly by eating marine animals found in surrounding coastal waters as source of protein (Dede *et al*., 2002; Farombi *et al*., 2009).

The scarcity of petroleum product in Nigeria sometimes in the years gone by and the booming black

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market dealing in refined crude oil has led to the situation whereby petroleum product has continually been exposed to the human system via orogastric passage or accidental ingestion especially by the use of rubber hoses to suck fuel from car tanks into various categories of container (WWF, 2002). Kerosene was widely used in oil lamps and was one of the most important refinery products. It is used to power jet engine air craft (jet fuel) and some rockets but is also commonly used as a heating fuel and fire toys. It is also for effective killing of bed bugs.

Diesel is one of the fuels that can refine from crude oil. It is consider as a mid weight product it is obtained through the partial distillation of crude oil and diesel fuel is ignited in an internal combustion. It also contains higher quantities of mineral compound and sulfur. It is also called fossil diesel it is produced from the fractional distillation of crude oil (Chevron, 2003; Wellington and Asmus, 1995).

Petrol is produced in oil refineries and is one of the sources of pollutant gas. It consists mostly of aliphatic hydrocarbons. It is used as a solvent mainly known for its ability to dilute paints. It also used as vehicle fuel and in kitchen ranges and for lighting. It is used before as a treatment against lice and their eggs, this treatment method is no longer common because of the inherent fire hazard and the risk of dermatitis. It serves as cleaning fluid to remove stains from clothing (Chris, 2007).

Haematological effect on Albino Wistar rats exposed to crude oil polluted water were investigated at varying levels with shellfish which had been previously exposed to crude oil polluted water and the oral gavaging with crude oil at the rate of 3, 6 and 9 mL kg^{-1} body weight per day. The resulted changes in Packed Cell Volume (PCV), Red Blood Cell (RBC) and White Blood Cell (WBC) counts and Haemoglobin concentration (Hb) of rats were significant ($p<0.05$). This results in haematoxicity (Eyong, 2000).

The effects of the ingestion of crude oil contaminated feeds on the yield and quality of egg of poultry birds were observed by Ogbalu. The weight of the eggs of the control were significantly ($p\leq0.05$) higher than the treated cases. The mean albumen height also varied significantly. The effects would collectively affect the quality of the egg, survival of the embryo and their hatchability.

The antioxidant systems of the testes and epididymal sperm in rats by oral exposure to 0, 200, 400 and 800 mg kg^{-1} bonny light crude oil showed that the testes and sperm at all doses were significantly ($p<0.05$) affected. The testes were characterized by severe congestion of interstitial vessels, decreased germinal epithelium and increased number of vacuolization (Farombi *et al*., 2009). Also Orisakwe *et al*. (2004) observed the treatment of male albino rats with 200, 400 and 800 mg kg^{-1} body weight bonny light crude oil to showed a dose-dependent decrease in the absolute weight of the testes. Histological evaluations of the testes showed slight to severe degeneration or even complete absence of seminiferous tubules and necrosis of cells depending on the dose of the crude oil. Exposure of rats to Nigerian Qua Iboe Brent crude oil via oral administration of increasing doses every day for 4 weeks showed a significant ($p<0.01$) dose-dependent reduction in the caudal epididymal sperm reserves of rats that received crude oil treatment relative to the control group. The morphology of testes of the crude oil exposed rats was characterized by the presence of interstitial exudates, degeneration and necrosis of spermatogenic and interstitial (Leydig) cells (Obidike *et al*., 2007; Adesanya *et al*., 2009).

MATERIALS AND METHODS

Total 25 healthy male Wistar rats of weight between 93-230 g were obtained from the animal house of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

The rats were acclimatized for 14 days within the experimental animal unit and fed with rat commercial rat cubes (SF Feeds, Nigeria Ltd.). They were allowed unrestricted access to clean fresh water dispensers *ad libitum*. The rats were randomly divided into 5 groups (A-E) containing 5 rats each. Group E served as control and was giving distilled water instead of the hydrocarbons.

The petrol and diesel products were obtained from total petroleum filling station, Ogbomoso, Nigeria. Groups A and B were orally dosed with 0.2 and 0.4 mL/rat of petrol, respectively likewise groups C and D were dosed with 0.2 and 0.4 mL/rat of diesel. The dosing was done every day for 28 days.

About 5 h after the last dosing on the 28th day, all the rats were sacrificed by cephalic dislocation, the testicles and epididymides were harvested through a lower abdominal incision. The epididymides were separated from the testes by blunt dissection. The epididymides were cut open longitudinally and with gentle pressure on the serosa a drop of semen was expressed on a pre-warmed (37°C) slide. Semen examinations were done using methods described by Zemjanis (1970). Briefly, a drop of sodium citrate buffer

(2.9%) was added to the expressed semen and cover slip was applied to evaluate motility under x40 of microscope. The semen sample was also stained with Eosin-Nigrosin to evaluate live-dead ratio. This same sample was used to estimate sperm abnormalities. The epididymides were then submerged in a graduated test-tube containing 5 mL of formol saline. Semen volume was roughly evaluated as the measure of displacement of formol saline. The entire epididymis was then crushed in formol saline and this mixture was used to evaluate spermatozoa concentration using the improved neubar chamber. Following separation of the epididymides, the testicles were fixed in Bouin's fluid in labelled bottles. Tissues were processed routinely and embedded in paraffin wax as described by Avwioro (2002) (Fig. 1).

Statistical analysis: Data obtained were expressed as Mean±SEM. A two way Analysis of Variance (ANOVA) was employed in analyzing the data. Duncan's multiple range t-test was carried out to determine statistical significance between treatment means at 95% confidence level. The tests were considered statistically significant when $p<0.05$. Graphpad Prism software in 2009 edition was used.

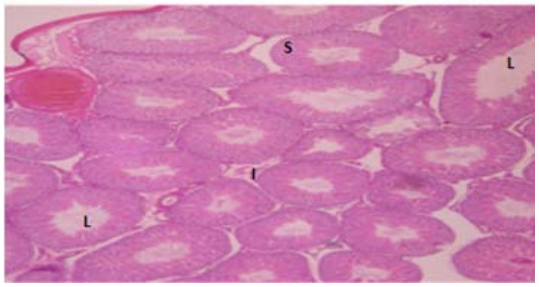

Fig. 1: Transverse section of Testes (H and E x100), dosed 0.2 mL distilled water (Control) showing Lumen (L), Interstitial cell (I) and Sertoli cell (S)

RESULTS AND DISCUSSION

There was no significant change on body weight of the rats dosed petrol and diesel when compared with the control group (Table 1). The effects of diesel and petrol on sperm parameters of Wistar rats was shown on the Table 2, the difference in sperm counts was not statistically significant ($p>0.05$) when compared the two hydrocarbons with the control.

The motility of the spermatozoa was highly affected by the two hydrocarbons the percentage of motile sperm in the control rats was far higher (84.58%) than the percentage of motile sperm in the rats treated with petrol (69 and 36%) and diesel (36 and 46%), respectively. The statistical difference was significant ($p<0.05$) in dose related manner. The percentage of normal sperm cells was adversely reduced ($p<0.05$) in hydrocarbon treated rats, petrol (38 and 27%) and diesel (32 and 24%) when compared with the control (83.08%). The rats treated with diesel and petrol (0.2 and 0.4 mL/rat) exhibited histopathological effects in a dose related manner (Fig. 2-5). The petrol treated rats at 0.2 and 0.4 mL showed seminiferous tubules with absence of lumen, distrupt

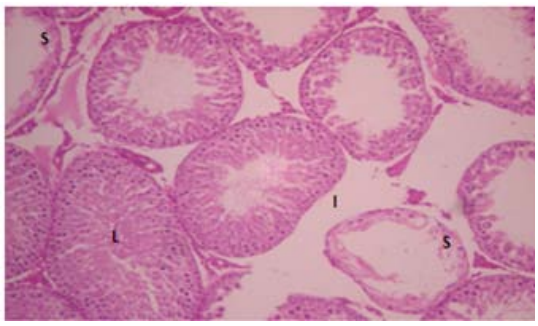

Fig. 2: Transverse section of testes (H and E x100), dosed 0.2 mL petrol showing seminiferous tubules with absence of Lumen (L), degenerated Sertoli cell (S) and Interstitial cell (I)

Table 1: Weekly body weight of the rats in Mean±SEM

		Petrol (mL)		Diesel (mL)	
Period (weeks)	Control	0.2	0.4	0.2	0.4
1	124.84±2.02	136.04±2.33	152.68±2.27	190.82±5.34	216.02±3.76
2	124.96±2.04	135.78±2.45	152.52±2.32	188.88±5.02	215.00±3.33
3	125.06±2.08	135.86±2.34	152.04±2.36	189.89±5.49	215.88±3.81

Table 2: Mean±SEM of sperm parameters

		Petrol (mL)		Diesel (mL)	
Sperm parameters	Control	0.2	0.4	0.2	0.4
Sperm count ($\times 10^6$)	20.00±6.7	18.98±1.56	18.34±0.99	18.10±2.12	18.68±1.74
Motile sperm (%)	84.58±2.9	69.00±7.40*	36.00±5.10*	38.00±3.74*	46.00±4.00*
Normal sperm (%)	83.08±3.1	38.00±9.10*	27.00±3.00*	32.00±3.74*	24.00±9.27*

*$p<0.05$; otherwise $p>0.05$

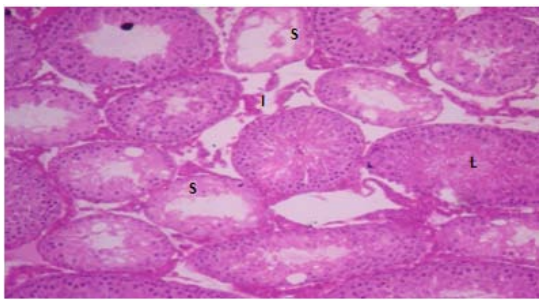

Fig. 3: Transverse section of testes (H and E x100), dosed 0.4 mL petrol showing shrink seminiferous tubules, absence of Lumen (L), Interstitial cell (I) and Sertoli cell (S)

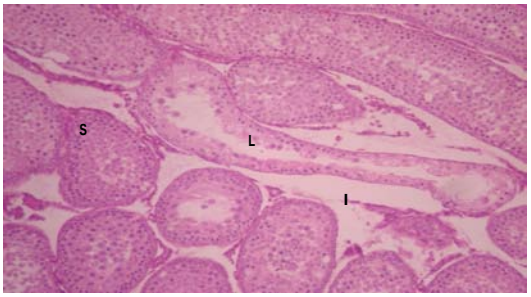

Fig. 4: Transverse section of testes (H and E x100), dosed 0.2 mL diesel showing shrink seminiferous tubules, absence of Lumen (L), Interstitial cell (I) and Sertoli cell (S)

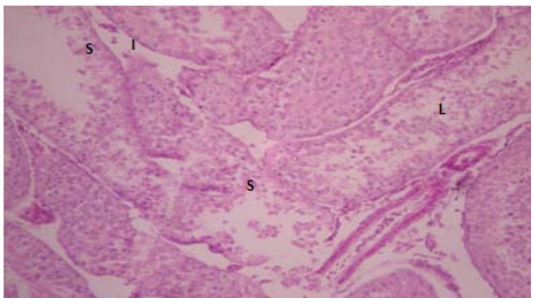

Fig. 5: Transverse section of testes (H and E x100), dosed 0.4 mL diesel showing absence of Lumen (L), degenerated Sertoli cells (S) and elongated seminiferous tubules

histoarchitecture of seminiferous tubules with vascular congestion of the interstitium and prominence of the interstitial cells (Fig. 2 and 3). Meanwhile, the diesel treated rats at 0.2 and 0.4 mL/rat exhibits shrunk, conjoin and elongation of seminiferous tubules with absence of lumen and lost of sertoli cell (Fig. 4 and 5).

The body weight gain of the rats treated with bonny light crude oil was significantly higher than the control (Adesanya *et al*., 2009). However, this was in contrary to the present observation which showed insignificant ($p>0.05$) increase in body weight of the rats treated with petrol and diesel. Researchers observed that the petrol and diesel, some of the major constituents of crude oil was discovered to posed reproductive damages on male rats, these effects includes significant reduction ($p<0.05$) in the number of sperm cells, increase percentage of abnormal spermatozoa with respect to both hydrocarbons compared with the control rats and statistically decrease in the amount of motile sperm. The abnormal morphology is frequently associated with low sperm counts and poor sperm motility but it may also be a primary factor when other measurements are normal. However, the abnormal sperms are incapable of fertilizing eggs because they lack acrosomes.

Thus, the findings corroborates previously reported studies that Polyacromatic Hydrocarbons (PAHS) of crude oil have adverse effects on male reproductive system such as testicular changes like wasting with lack of sperm cells (Eyong, 2000) declining trend in the sperm count of man and wildlife animals over the past decades disruptions of sex hormones and induced reproductive toxicity (WWF, 2002). Severe congestion of interstitial vessels, decreased germinal epithelium and increased number of vacuolization was reported (Farombi *et al*., 2009). Also Orisakwe *et al*. (2004), Obidike *et al*. (2007) and Adesanya *et al*. (2009) observed the treatment of male albino rats with bonny light crude oil to showed slight to severe degeneration or even complete absence of seminiferous tubules and necrosis of cells depending on the dose of the crude oil. Meanwhile in the present study on petrol and diesel there was sertoli cells degeneration, shrinking, elongation and congestion of seminiferous tubules with no luminal space and wider interstitial cell of leydig.

CONCLUSION

The results of this study contribute an important toxicology perspective to findings on petrol and diesel exposure in the environment. It was obvious that these hydrocarbons were spermatotoxicity and testotoxicity in male rats.

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