

EFFECTS ON TESTES AND EPIDIDYMAL SPERM IN MALE WISTAR RATS FOLLOWING ORAL ADMINISTRATION OF KEROSENE AND PETROL

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ABSTRACT

Crude oil is a complex mixture of many different components. Some of the crude oil components; petrol, kerosene and diesel are consumed by man for the management of various forms of gastrointestinal problems and male productive capacity either directly as curative agents or indirectly by eating marine animals.

This study investigates the effect of kerosene and petrol on testes and sperm parameters using twenty-five male Wistar rats divided into 5 groups (n=5). A control group received 2ml distilled water, 2 petrol groups of rat and 2 kerosene groups of rat both were orally dosed with increasing doses of 0.2ml and 0.4ml respectively every other day for 28 days.

The test rats were restless and sniff repeatedly during the second week of the treatment. The mean body weight of the rats was not statistically ($P > 0.05$) affected in both kerosene and petrol test rats.

The caudal epididymal sperm was insignificantly decreased in number and there was reduction in daily spermatozoa production. The statistical values of sperm motility were significantly ($P < 0.05$) decreased, while the statistical relationship of the motile sperm between

the two hydrocarbons was not significant. However, total sperm abnormalities were significantly ($P < 0.05$) increased at all dose levels.

The seminiferous tubules are reduced in size, with vascular congestion of the interstitium and prominence of the interstitial cells of Leydig. The lining cells of the spermatogenic series are reduced, and there is paucity of luminal spermatids.

These effects would collectively affect the quality of the sperm and performance of the testes which may contribute infertility in male.

{**Citation:** Akintunde O. W., Ojo O. A., Olaniyan L.W. B., Kehinde, B. D. Effects on testes and epididymal sperm in male wistar rats following oral administration of kerosene and petrol. American Journal of Research Communication, 2013, 1(8): 148-159} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

All life required crude oil to make each day complete. Crude oil has become the world's most important source of energy since the mid 1950's and it has proved to be useful to the whole world (Hyne, 2001). Crude oil is the term for "unprocessed" oil, the stuff that comes out from the decaying plants and animals living in ancient seas millions of year ago. It is also called a fossil fuel because it was formed from the remains of tiny sea plant and animals died; they sank to the bottom of the oceans (Okoh, 2003). Crude oil is separated by a process which is known as fractional distillation. Crude oil is basically heated to very high temperature until the hydrocarbons that make up the crude oil separate into different distillates; jet fuels, gasoline, kerosene, light virgin naphtha, petroleum spirit, petroleum naphtha, diesel fuel oil as well as most domestic fuels and light crude marine gas oils. Crude oil is the mains stay of the Nigerian economy and constitute about 90% foreign exchange earning of the nation. Apart from the

financial benefits, the exploration of crude oil brings about the pollution of our environment including our waterways (rivers and streams). Hence, crude oil exposure presents a potential hazard to both aquatic and terrestrial species. This exposure may occur during extraction, refining, transportation and utilization (Thomas, 2000).

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 (Ebenezer, *et al*; 2009). In our environment, crude oil components are capable of mimicking the inherent actions of reproductive hormones and have the ability to disrupt the neuroendocrine system or the function of the gonads directly (Veeramachaneni, 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, 2002. The weight of the eggs of the control were significantly ($p < 0.05$) 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 800mg/kg 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 (Ebenezer, *et al*; 2009). Also, Orisakwe *et al*, 2004 observed the treatment of male albino rats with 200, 400 and 800mg/kg body weight bonny light crude oil to show 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 (0.1, 0.2, and 0.4 ml/rat) every other 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 (Reginald *et al*; 2007).

MATERIALS AND METHODS

Twenty-Five healthy male Wistar rats of weight between 93-230g were obtained from the animal house of Ladoké Akintola University of Technology, Ogbomoso, Nigeria.

The rats were acclimatized for 2 weeks. The rats were fed with rat pellet and water *ad libitum*.

The rats were randomly distributed into five (5) groups and the products were administered orally every other day for 28 days (table 1).

Table 1: Dosing of the rats

Groups	Number of rats per group	Dosage
1	5	0.2ml distilled water (Control)
2	5	0.2ml of kerosene
3	5	0.4ml of kerosene
4	5	0.2ml of petrol
5	5	0.4ml of petrol

Five hours after the last dosing, on the 28th day, all the rats were sacrificed by cervical dislocation; the testes and epididymis were harvested for routine histological analysis (Avwioro, 2002) and sperm analysis respectively.

Statistical analysis

Data were expressed as Mean \pm S.E.M. A two way analysis of variance (ANOVA) was employed in analyzing the data. Duncan's multiple range t-tests was carried out to determine statistical significance between treatment means at 95% confidence level. The tests were considered statistically significant when $P < 0.05$. The software was Prism Graphpad, 2009 edition.

RESULTS

EFFECTS ON MEAN BODY WEIGHT

There was no significant effect on body weight of the rats dosed kerosene and petrol when compared with the control group (Table 2).

Table 2: Weekly Body weight of the rats in Mean \pm S.E.M

	DISTILED WATER	KEROSENE		PETROL	
Period	Control	0.2ml	0.4ml	0.2ml	0.4ml
Week1	124.84 \pm 2.02	136.04 \pm 2.33	152.68 \pm 2.27	114.06 \pm 0.77	118.62 \pm 0.80
Week2	124.96 \pm 2.04	135.78 \pm 2.45	152.52 \pm 2.32	114.48 \pm 0.59	118.38 \pm 1.30
Week3	125.06 \pm 2.08	135.86 \pm 2.34	152.04 \pm 2.36	113.54 \pm 0.70	119.56 \pm 0.90

SPERM PARAMETERS

The sperm parameters showed diverse changes within the dosed level with the two variables. The sperm count of rats dosed with kerosene showed slight decrease in number, the decreased was not statistically significant ($P > 0.05$); the same applied to petrol dosed rats (Table 3). When compared the two variable (kerosene and petrol), the number of sperm cells showed no significant different ($P > 0.05$) (not shown in the table). The motility effects of the cells were clearly shown in table 3, the percentage of motile sperm cells was statistically decreased ($P < 0.05$) for both kerosene and petrol when compared with the control in dosed dependent manner. Meanwhile, the statistical evaluation between the variables (kerosene and petrol) showed no significant different ($P > 0.05$). Except at high dose (0.4ml/rat) where the kerosene dosed rats had 54.00 ± 10.5 value higher than the petrol dosed rats (36.00 ± 5.1) at the same dose.

Morphologically, the mean percentage of normal cells in the control rats was high (83.08 ± 3.1) while the treated rats had a significantly low percentage ($P < 0.05$) for both variables. Meanwhile, at 0.4ml kerosene the mean percentage of normal cells was 42.00 ± 12.0 higher than the rest of the treated rats, the lowest mean percentage for kerosene groups was at 0.2ml (16.00 ± 2.5) while the petrol lowest mean percentage was at 0.4ml (27.00 ± 3.0) (Table 3).

Histopathologic observation

Normal histology of the testes was shown in plate 1, characterized by normal seminiferous tubules with lumen, interstitial cell of Leydig and sertoli cells. The treated rats histology for both hydrocarbons; kerosene (Plates 2 and 3), and petrol (Plates 4 and 5) showed similar features which characterized by reduction in size of seminiferous tubules, vascular congestion of the interstitium and prominence of the interstitial cells of Leydig. The lining cells of the spermatogenic series are reduced, and there is paucity of luminal spermatids.

Table 3: Mean \pm S.E.M of Sperm Parameters

	Control	KEROSENE		PETROL	
		0.2ml	0.4ml	0.2ml	0.4ml
Sperm Count ($\times 10^6$)	20.00 \pm 6.7	17.60 \pm 2.3	19.00 \pm 1.2	18.98 \pm 1.6	18.34 \pm 0.9
Motile Sperm (%)	84.58 \pm 2.9	44.00 \pm 2.5*	54.00 \pm 10.5*	69.00 \pm 7.4*	36.00 \pm 5.1*
Normal Sperm (%)	83.08 \pm 3.1	16.00 \pm 2.5*	42.00 \pm 12.0*	38.00 \pm 9.1*	27.00 \pm 3.0*

*P < 0.05, otherwise P > 0.05

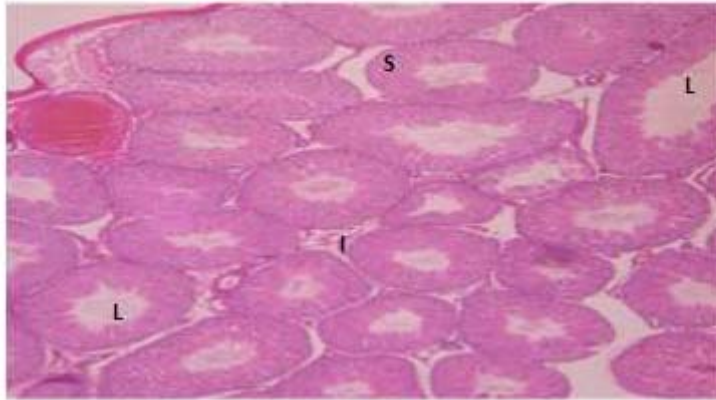


Plate 1: Transverse section of Testes (H&E x100), dosed 0.2ml distilled water (control), Showing lumen (L), interstitial cell (I) and sertoli cell (S).

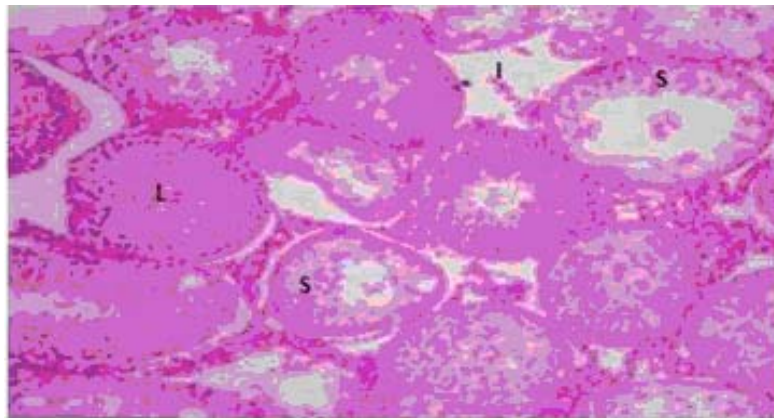


Plate 2: Transverse section of Testes (H&E x100), dosed 0.2ml kerosene, Showing Seminiferous Tubules with absence of lumen (L), interstitial cell (I) and degeneration of sertoli cell (S).

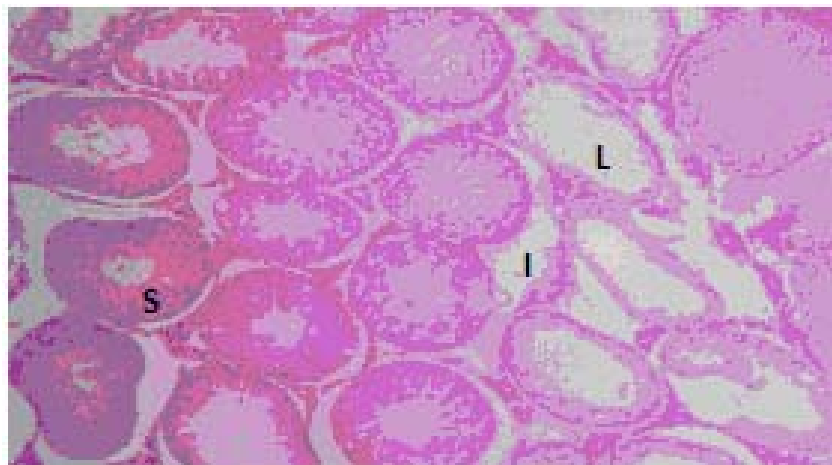


Plate 3: Transverse section of Testes (H&E x100), dosed 0.4ml kerosene, showing reduction size of seminiferous tubules with lumen (L), interstitial cell (I) and degenerated sertoli cell (S).

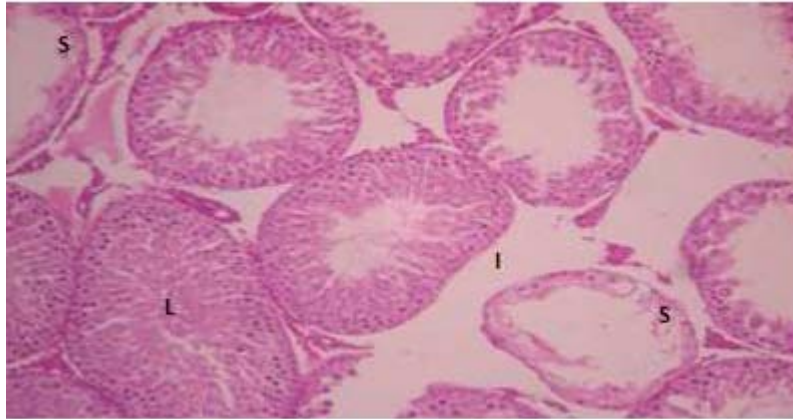


Plate 4: Transverse section of Testes (H&E x100), dosed 0.2ml petrol, showing seminiferous tubules with absence of lumen (L), degenerated sertoli cell (S) and interstitial cell (I).

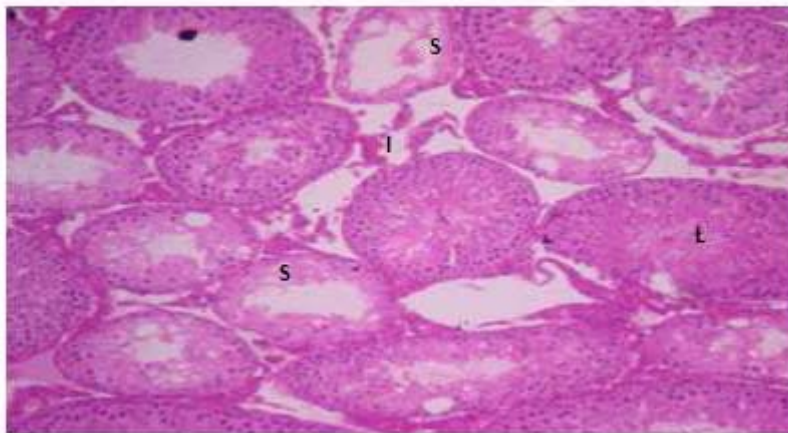


Plate 5: Transverse section of Testes (H&E x100), dosed 0.4ml petrol, showing shrink seminiferous tubules, absence of lumen (L), interstitial cell (I) and sertoli cell (S).

DISCUSSION AND CONCLUSION

Data from the present work showed that body weight of the treated rats was not statistically affected contrary to previous report by Adesanya *et al*, 2009. In the present study the decrease in the total number of sperm cells was not significant, which shows that the hydrocarbons has no significant effect on sperm production thus, there may be other contributing factor(s) that caused the insignificant reduction in number of epididymal sperm. Meanwhile, Ebenezer, *et al*; 2009, has contrarily reported a significant decreased in number of sperm count in laboratory rats, However, the percentage of the motile sperm of the treated rat was significantly reduced when compared to the control; similar findings were seen in previously reported studies (Ebenezer, *et al*; 2009). Decrease in the percentage of normal sperm with decrease in the number of motile sperm and sperm count, testes with slight to severe degeneration or even complete absence of seminiferous tubules and necrosis of spermatogenic and interstitial (Leydig) cells depending on the dose have most frequently been observed as one of the earliest indication of testicular pathology, Thus, the findings of the present work may promote infertility by altering the function of the testes and sperm, particularly by way of induction of oxidative stress, these were also evident in previously reported work (Orisakwe *et al*, 2004; Ebenezer, *et al*; 2009; Reginald, *et al*, 2007).

We conclude that with the result of this study the hydrocarbons (kerosene and petrol) were toxic to the sperm parameters and testes of the rats, these may promote infertility in male by altering functions of the testes or imply possible reproductive health hazards for animals and humans that may be exposed to this environmental pollutant, especially in areas where oil spillage is a common phenomenon.

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