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Acute Toxic Effects Of Two Grades Of Diesel Fuel Oil On Rat Lungs

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ABSTRACT

A total of seventy (70) Albino Wister rats of both sexes with an average weight of 200g were used for the study. Single varying doses of two grades of diesel fuel oil were injected intra-peritioneally to determine (a) the LD_{50}, and (b) the toxic pulmonary effects. Results show the LD_{50} for pure diesel oil to be 37.18g/kg and that for adulterated diesel oil to be 36.98g/kg. Results show a dose dependent effect on the breathing pattern with breathlessness increasing in severity with increasing doses. Histopathologic examination of the lungs show a dose dependent thickening of the alveolar septa due to edema, inflammatory cell infiltrates. In those animals subjected to very high doses of diesel oil, there is evidence of pulmonary hemorrhage and disruption of the alveolar septa walls.

Key Words: Diesel, rat lungs, breathing.

Petroleum industry has become the mainstay of Nigeria economy, contributing enormously to physical development of infrastructural facilities such as rural electrification, road construction, hospitals and schools.

Inhabitants of oil producing areas believe that petroleum samples are of some medical values as it is said to stop or reduce the effect of poison i.e. as in snake antidote, as emetic against ingested poison and also for pain relief in arthritic condition and anti- convulsant effect.

Although petroleum exploitation has improved mechanized agriculture, Nigerian's economy and medical values (NNPC, 1979), however much dependence has also increased cases of environmental pollution and this is detrimental to human existence (NRC, 1976; Ruddel *et al*, 1994).

Most toxicity studies using experimental animals always report that there is significant health risk following a prolonged exposure to diesel exhaust emission (Sheepers & Bros, 1992).

Consequently, fuel has shown acute toxicity when administered through oral, demal and inhalation routes to laboratory animals (in vivo) test system. However, findings on embryotoxicity, teratogenicity, mutagenicity and genotoxicity were found to be negative (WHO, 1997).

Toxicity of petroleum has also been identified by a function of its two or three ring aromaticity in napthalenes and anthracenes (Anderson, 1974) with diesel fuel, the absolute amount of these aromatics is high (Kuhnhold *et al*, 1980)

Based on various works and reports published on the effects of petroleum sample on the environment, no report on the comparative study has been published on the deleterious effect of adulterated diesel fuel and pure diesel fuel on rat lungs. Hence this study investigates the acute toxicity effects of two grades of diesel fuel on rat lungs.

MATERIALS AND METHODS

Material:

A total of 70 rats (*Ratus ratus*) sixty-six males and four females of weight range 200-280g were used in this study. The animals were obtained from Phamacology Department and Animal House of the Faculty of Biological Sciences of the University of Port-Harcourt.

The rats were put in cages and were fed with feed and water *ad labitum* for

about 18 days to acclamatize them to the new environment.

Two grades of diesel fuel (pure and adulterated) were used in this study. The pure diesel was obtained from an Agip filling station in town while the adulterated diesel fuel was obtained from a local source in Port-Harcourt.

Procedure

A pilot study was carried out on ten rats by injecting random doses of fuel in order to determine the smallest dose that could kill the rats within 24 hours, and consequently, the dose that neither killed nor elicited any observable sign of toxicity within the same period. the study was carried out concurrently with both adulterated and pure diesel fuels using 5 animals each.

The remaining rats were then divided into another two groups each for both the adulterated and pure diesel fuels. Each of the groups was further divided into six groups with five rats in a cage.

The two groups were injected intra-peritioneal with doses of 10.0g/kg, 21.7g/kg, 43.5g/kg, 65.25g/kg and 87.0g/kg of both pure and adulterated diesel fuel respectively, while 65.25g/kg of pure diesel was administered to just the pure diesel fuel group only.

The doses correspond to 2.5mls, 5mls, 20mls 15mls and 40mls of these fuel. The groups injected with 20mls of 0.9% Saline and water served at the control groups.

The subjects received injection intra-peritioneally every morning and then observed over a period of 24hrs. Rats from the group administered with the least dose that killed all the animals in a group (LD_{100}), the control groups and all other groups were sacrificed in the 24th hour and 48th hour using chloroform anaesthesia. Their lungs were removed and fixed in 10% formalin for histological studies. The fixed tissues were processed histologically, and sections stained with Haematoxylin and Eosin.

Clinical Signs And Mortality

The numbers of dead animals per group within 24 hours were recorded. This information was also useful for estimation of the median lethal dose LD_{50} of the diesel fuel in rats using arithmetic method of Karber (Dede and Igbigbi, 1997). However, some of the animals especially those with higher doses were reported dead when they no longer responded to prodding and agitation.

RESULT

Acute toxicity test and Behavioural pattern

It was observed that the their was a dose dependent reduction of activity and feeding of the rats administered with different dosages of fuel. These reflected in relative loss of appetite to cessation of feeding for 43.5g/kg and 87g/kg dose groups of fuel.

The injection /treatment related clinical effects include: changing of the eyes to deep pink colour, laboured breathing, lack of coordination, dizziness, weakness, abnormal swelling and Ataxia. While the control groups showed signs of weakness after which they became normal. However, all the animals that received 87g/kg of these diesel fuel died within 24 hrs.

The abnormal changes in the behavioural patterns were also dose dependent. Animals with lower dosages 10.9g/kg, 21.75g/kg showed some slight reduction in movement from one edge of the cage to the other but were relatively quiet while the higher dosage showed slight movement even on agitation and prodding. These animals also passed out frequent pink but little urine.

Table 1: LD_{50} Determination For Adulterated Diesel Fuel

S/n	N0 in Cage	Normal Saline (ml)	Dosage (gkg^{-1}) Diesel	Dose Difference (gkg^{-1})	No Alive	No Dead	Mean Dead	Mean deas & dos diff (gkg^{-1}).
1	5	0	0	0	5	0	0	0
2	5	20	0	0	5	0	0	0
3	5.	0	10.90	10.90	4	1	0.5	5.45
4	5	0	21.75	10.85	3	2	1.5	16.28
5	5	0	43.50	21.75	2	3	2.5	54.38
6	5	0	87.00	43.50	0	5	4.0	174.00
							TOTAL	**250.11**

LD_{50} = least dose that killed 100% of animals

$$\frac{\text{Summation of mean dead (M.D.) x dose difference}}{\text{No of animals per group}}$$

$$= 87 - \frac{250.11}{5} = 87\ \ 50.02 = 36.98gkg\text{-}1$$

LD_{50} = 36.98gkg-1.

Table 2: LD_{50} For Pure Diesel Fuel

S/n	N0 in Cage	Dosage (gkg^{-1}) Diesel	Dose Difference Diesel	No Alive	No Dead	Mean Dead	Mean deas & dos diff (gkg^{-1}).
1	5	0	0	5	0	0	0
2	5	10.90	10.90	4	1	0.5	5.45
3	5	21.75	10.85	3	2	1.5	16.30
4	5	43.50	21.70	2	3	2.5	54.35
5	5	63.30	21.80	1	4	3.5	76.30
6	5	87.00	21.70	0	5	4.5	96.70
						TOTAL	249.10

$$LD_{50} = {}_{87}\ \frac{249.10}{5}$$

$$= 87\ \ 49.82$$

$$= 97.18gkg^{-1}$$

Fig. I: Thickening of the alveolar septa and membrane Black deposits of inflammatory cells are seen Mag - X 400

Fig. 2: Control Showing
As - Alveolar Septa
AI - Alveoli
BV - Blood Vessels
Mag - X 400

Fig. 3: Tissue Necrosis with complete destruction of the alveolar septa Mag - x 400

Fig. 4: Thickened alveolar wall complete destruction of the alveolar septa deposits of black material (inflammatory cells) and also pulmonary haemorrahage
Mag - X 400

Histopathology

Results showed a dose dependent thickening of the alveolar septa with pulmonary oedema and pneumonitis (Fig 1). This reaction occurs with both the pure and adulterated diesel fuel. Other histopathologic findings include pulmonary haemorrhage (Fig. 3), and tissue necrosis (Fig. 4), in those rats administered with the highest doses of the sample fuel. Dose dependent inflammatory cell deposits of black substances are seen (Fig. 1).

DISCUSSION

Intra-periotioneal administration of diesel fuel is a reliable means of toxicological studies in rats (Matsumura, 1975). The LD_{50} values of 37.18g/kg and 36.98g/kg for pure and adulterated oil respectively tends to confirm the relative low toxic nature of diesel fuel oil (WHO, 1997; Kuhnhold *et al.*, 1980).

Toxic pulmonary effects of petroleum products have already been documented (WHO, 1997; Kuhnhold *et al.*, 1980; Wolf *et al.*, 1996). These studies showed that death due to petroleum product poisoning is usually ascribed to hypoxia xaused by atelectasis than to damage to other systems like the kidney, liver, and the gastrointestinal tract. This tends to agree with the pulmonary changes seen in this study.

The deposition of black substances in the lung tissue or interstitium as seen in this study has been attributed to lipoid pneumonia resulting from petroleum product poisoning (Becklake, 1997).

The results tend to confirm that though diesel oil has low toxicity as seen by the LD_{50} values, however, the toxic effects produced are similar to that of other petroleum products, and are particularly on the respiratory system.

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