



## A Study On The Acute Toxicological Effects Of Commercial Diesel Fuel In Nigeria In Rats (*Ratus ratus*.) Using Hematological Parameters.

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**ABSTRACT:** The acute toxicology effect of diesel fuel in rats using hematological parameters was investigated. Five groups of rats consisting of ten rats per group, all of 0.2kg average body weight were used for the study. Four doses (65/kg; 87 g/kg; 209; g/kg; 131 g/kg) of diesel fuel were administered intraperitoneally, into the rats and the effect monitored within 24hours. The following hematological parameters ( Haemoglobin concentration, palled cell volume, white blood cell count) and enzyme (Aspartate/ alamine aminotransferases and alkaline phosphatase) levels were monitored. Results indicated a decrease in haemoglobin concentration, packed cell volume and white blood cell count with increase in dose levels of diesel fuel in the rates. Conversely, there was an increase in serum enzyme levels with increase in dose of diesel fuel.

The results indicate possible aplastic anaemia in the rat being in the rats being induced by administration of diesel fuel. Furthermore, the enzyme analysis results suggested a possible hepatotoxic effect of diesel fuel in the rat. @ JASEM

The toxicity of petroleum products in general, to human and laboratory animals depend on certain physical properties of the products such as their viscosity and surface tension, in addition to the chemical properties of individual products. A low viscosity enables a deeper penetration of fluid into the distal airway and other tissues, which a low surface tension enhances spread over the surface contacted (Ervin, 1983). A study of the transfer of petroleum solvents through the placenta in pregnant woman showed that solvent concentration in the blood of the embryo and the newborn were approximately twice that in the blood of the mother (IPCS, 1982). Report furthermore, report indicate that benzene (aromatic hydrocarbon) content of gasoline as k known to induce acute non-lymphocytic leukaemia though occupational exposure (Austinetal, 1988), the aromatic content of gasoline is about 10% of its hydrocarbon composition (Frankeriberger and Johanson, 1982), Diesel fuel, a broad mixture of hydrocarbons with 12-20 carbon atoms per molecule and a distillation range of 200-380<sup>0</sup> C (Speight, 1992), has a 25% aromatic content (Frankenberger and Johanson, 1982). An evaluation of toxicity studies in laboratory animal and *in vitro* test system concludes that diesel fuel has how acute toxicity when administrated via oral, dermal and inhalation routes (WHO, 1997), however, with a substantial aromatic content the pathophysiological effect of diesel fuel may be high, since the aromatic dose groups were centrifuged at 12000g for 10mins using a special speed automated microhaematoorit centrifuge (baker

& Silverton, 1985). The packed cell volume was subsequently determined by meaning the height of the red cell column and using a PCV reader.

$$Le = \frac{\text{Height of Red cell column}}{\text{Height of Total column.}}$$

### *White Blood Cell Count.*

The anticoagulated (citrated) blood was diluted with 2% acetic acid (20ml/L) tinged with gentian violet? This was to lyse the red blood cells and also stain the white cell nuclei.

A final dilution of 1: 20 was made before loading it into an improved Neubauer counting chamber and counted under the light microscope (Baker and Silverton, 1985). Using this counting chamber, white calls in the four corner Imm<sup>2</sup> areas and those in the central 1 mm<sup>2</sup> areas were counted. Precautions such as filtering the diluting fluid before dilution of the blood was taken to prevent dust and debris interfering with the accuracy of the count.

### *Enzyme Assay*

The blood serum obtained from the coagulated blood was assayed for enzymes which provided information on the hepatocellular integrity and biliary tract function ie the aminotransferases and alkaline phosphatase enzymes respectively fraction of fuels is known to have the highest physiological impact (Kiihnhold, 1980). The current that before is aimed at studying the possible acute toxicity effect of

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commercial used fuel in Nigeria on Blood of white albius rats

## MATERIALS AND METHODS

Two types of blood sample test tubes were used for this analysis; the citrated and non-citrated test tubes. The citrated test tubes were used to collect non-coagulated blood needed for haemoglobin concentration, packed cell volume and white blood cell analysis; the non-citrated test tubes allowed for coagulation, to obtain the blood serum needed for enzyme assay.

### Haemoglobin Concentration

The method used in this analysis estimated the haemoglobin concentration by light absorption at 540nm as reported by strove and Makarova (1989). 8ml of ammonium hydroxide was measured into a test tube using a micropipette. The micropipette was then used to suck up and pour back the solution three times, then the entire solution was mixed thoroughly by shaking. A clearing up of the solution indicated a complete laemolysis. The test tube was further shaken for three minutes to ensure a complete oxygenation of the haemoglobin.

The absorbance of the haemolyzed blood solution was measured against distilled water on a photocolormeter at 540nm (green light filter) using 0.5cm thick cells. The values obtained for the blood from diesel-treated rats ere recorded against that from the control animals. This method presumes the

intensity of absorbed high to be proportional to haemoglobin concentration.

### Packed Cell Volume (Pcv)

The citrated blood of the rats from the various phosphatase respectively.

Alanine and Aspartate aminotransferases (alat: ASAT)

These enzymes were assayed using the Reitman and Frankel method as described by strove and Makarova (1989): Dede (1992). In these method, the amount of pyruvic acid produced from AIAT - catalyzed reaction and from oxaloacetate decarboxylation by As AT- catalyzed reactions, is determined by its colour reaction with 2,4 dinitrophenylhydrazine.

The absorbance for sample solution was measured against control solution on a photocolormeter at 500-560nm (green-light filter) using 1 cm thick cells. The enzyme activity is calculated using the prescribed standard formula.

### Alkaline Phosphatase

The Bodansky method, involving the photocolormetric determination of inorganic phosphate cleaved from B-glycerophosphate by phosphatase of the blood serum in an alkaline medium, was used (Strove and Makarova 1989). In this method, enzyme activity was estimated from measuring absorbance on a photocolormeter at 630-690nm (red light filter) wavelength using 0.5cm thick cells.

**Table 1** Acute effects of intraperitoneal administration of diesel fuel on haemoglobin concentration, packed cell volume and white blood cell count of rats.

	Dose (g/kg)			diesel fuel	
	Control (0)	65	87	109	131
Haemoglobin (g/100ml)	12.3±0.17	9.4±0.21	5.3±0.10	3.6±0.15	3.3±0.20
Packed cell Volume (PCV%)	39±0.15	35±0.24	22±0.20	16±0.23	10±0.14
White Blood Cell count c.../mm <sup>3</sup> )	2600±9	2450±10	2300±6	1700±15	1400±11

**Table 2** Activity liver enzymes in blood serum of rats following a single in traperitoneal administration of diesel fuel.

	Dose (g/kg)			diesel fuel	
	Control (0)	65	87	109	131
Alanine aminotransferase	26.1±0.35	40.3±0.12	54.2±0.1	80.3±0.15	120±0.44
Aspartate aminotransferase	13.4±0.1	30.3±0.0.18	46.2±0.24	75.1±0.3	80.1±10
Alkaline phosphatase	25.2±0.32	30.5±0.11	31.3±0.2	41.4±0.14	45±0.32

For Tables 1 and 2  $n = 10, x \pm sem$

## DISCUSSION

The result indicated that increase in dose of diesel fuel administered into the rats caused a dose dependent decrease in haemoglobin concentration of diesel-treated rats. This finding was consistent with previous reports of the haemotocity effects of petroleum products (Stockman, 1977). There was also a decrease in packed cell volume and white blood cell count. This reduction in the haemoglobin concentration and the cellular constituents of the blood was suggestive of plastic anaemia (Brain, 1979). The induced anaemic condition in the rats was as a result of the low blood volume of diesel-treated rats, hence the difficulty in obtaining blood samples from these rats. During the experiment the aminotransferases are important enzymes which provide information on the integrity of hepatocytes. They are often membrane-bound enzymes in the hepatocytes, and hepatocellular injury may cause leakage of the enzymes into the serum. The marked elevation of the serum enzymes activities was therefore indicative of hepatic injury. Furthermore, there was also an increased level of serum alkaline phosphatase, an enzyme often used to estimate biliary tract function. The increase in serum activity of this enzyme suggested biliary tract dysfunction. Jeffries (1979) reported that effect of hepatotoxin in live injury to include pathophysiological symptoms, distinct biochemical changes manifesting as altered enzyme activity and/or biliary tract dysfunction. From the foregoing diesel fuel could be classified as a hepatotoxin diesel fuel is using not only to power motor vehicles, also electricity power generators in Nigeria. This no doubt increases the number of people that come into contact with diesel fuel. The possible toxicity effects of diesel in the current study have been highlighted.

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