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# The Effect of Gasoline Pretreatment in Diesel Toxicity of The Liver on Male Wistar Rats (Ratus Ratus)

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**ABSTRACT**

The inhalation route was used to assess the effects of gasoline pretreatment on diesel toxicity in rats. The intra-peritoneal $LD_{50}$ of gasoline, inhalational $LD_{50\ of}$ diesel and inhalational $LD_{50}$ of diesel following intra-peritoneal gasoline pretreatment in rats were obtained at 186.2 g/kg, 684 g/kg and 342 g /kg body weight respectively. Rats pretreated with gasoline showed marked signs of toxicity that included respiratory distress, sedation, coma and death. Those exposed to gasoline and diesel only were less affected. Liver enzymology showed liver damage with increased liver enzyme activity (ALT, AST, ALP) in all treated groups, but the effects were more pronounced in the gasoline pretreated group. This study suggests an enhancement of the toxicity of diesel by gasoline.

Key Word:Gasoline,Diesel, ALT, AST, ALP, Liver, Enzymology, Toxicity

## 1. INTRODUCTION

Diesel and Gasoline are common hydrocarbon products that can cause damage to the ecosystem when not properly controlled.In order to ascertain the extent of damage to humans who are normally exposed to interaction of these agents either as pump attendants at filing stations or mechanics who handle either chemicals or people exposed to adulterated forms which occur frequently in Nigeria, the animal experimental model was employed. Furthermore, since the pharmacokinetics of gasoline and diesel are poorly understood, the results of this research was thought could stimulate further studies and explain their interaction judging from the level of toxicity elicited against results of previous acute toxicity studies of individual substances and to determine if enhancement was important during interaction.

Large scale pollution by gasoline killed over 1000 persons in Delta State Nigeria[1;2]. Elsewhere in the world in Bahia Las Minas in Panamas in 1998 a spill of 3.8 million litres of diesel fuel occurred from a broken tanker [3]. In most cases pipeline vandalization and poorly maintained pipeline have increased land and water pollution by petroleum products especially as more than 4,200 kilometres in length of pipeline carry this products around Nigeria. Industrialization and development has greatly increased exposure to toxic hydrocarbon substances. Toxicity results from crude oil or end products. Toxicity disrupts normal metabolism of human cells. Petroleum hydrocarbons manifest their toxic effects by competing with some endogenous metabolites or block some pathways, this interference may be lethal [4].

Rats are usually used for human toxicological study. Acute toxicity study with experimental animals involves administering a single dose and observing its effects over a short period probably within 24 – 96 hours [5]. Urine and blood samples could be taken for biochemical tests. Also the animals could be killed and specific organs subjected to histopathological examination.

As diesel is a mixture of chemicals, the onset of local or systematic effects following dermal, oral or pulmonary exposure indicates that there are potential routes of absorption

  

for diesel. Occupational exposure may potentially occur during manual filling or discharge operations within petrochemical industry [6] repair or service of diesel engines or from practices where diesel is used as cleaning agent or solvent [7]. Large-scale environmental contamination has occurred following the release of diesel from storage tanks and sea tankers [7] and some concern has been expressed over health effects of vapour arising from contaminated soil [8]. Under normal conditions of storage, handling or use as fuel, diesel should not present a hazard to health providing excessive skin contact is avoided [9]. The main hazard associated with diesel is chemical pneumonitis that may arise following aspiration of vomitus (secondary to ingestion) or inhalation of aerosol (or aspiration of liquid during manual siphoning [10;11].

The liver is the seat of metabolism with thousands of enzymes with varying concentrations and half-lives. These enzymes are useful to estimate the liver function, with damage to liver cells leading to increase in the serum levels. Liver cell injury can be reasonably predicted when aminotransferase (transaminases) are elevated [12] especially in acute diseases. They have been referred to as hepatocellular enzymes [13]. Acute hepatocellular necrosis typically leads to elevated serum transaminases levels as a result of dying cells. Other features are variable and reflect the severity of damage [14].

There are few studies investigating the toxicity of diesel *per se.* Therefore, toxicological evaluation of diesel tend to be derived by considering the toxicity of similar (middle distillate products such as kerosene and petrol [7;10;11;15]. However, such comparisons do not take into account the toxicity of brand-specific additives, the effects of which cannot be predicted from complex hydrocarbon mixtures. One study has examined the effects of a combined exposure to diesel (5 ppm) and acetaldehyde (0.5 ppm) in Gulf War veterans but did not report any adverse effects in healthy volunteers [16].

No reports on the neurological effects of human diesel exposure were available in the literature. However, diesel is known to contain a number of potentially neurotoxic substances [15] and exposure to other mid-distillate fuels has resulted in neurological disorders including drowsiness, neurasthenia and decreased sensorimotor speed [10].

A practical consequence of interaction of two substances which have a different affinity for the same binding site is that the toxicity or efficacy of a substance which is normally highly bound will be increased, because the second substanceof greater affinity for the same sites will occupy the binding sites and so the free portion of the other substance reaches the toxic level [17]. The pharmacokinetics (ADME) of gasoline and diesel are not well studied and documented.

### 1.1 Aim and objectives of study

This research is geared toward assessing the effect of gasoline pretreatment in diesel toxicity of the liver on male wistar rats (Ratusratus)

**Objectives of Study**

The objectives are as follows:

1. To estimate theeffect of gasoline on liver enzymes of male Wistar rats
2. To determine the toxicity effects of varying doses of diesel on liver enzymes of male wistar rats
3. To evaluate the toxicological effects of Pretreatment with gasolineand then exposure to increasing concentration of diesel via inhalation route effects of Diesel on Liver Enzymes.
4. To use the histomorphological indices as a further assertions of the effects of gasoline and diesel on male wistar rats.

## 2.0 MATERIALS AND METHOD

### 2.1 EXPERIMENTAL ANIMALS

Male albino rats *(Ratusratus),* seventy (70) in number were sourced from the Biochemistry Department, University of Port Harcourt animal house. The animals were kept for fourteen (14) days in the

  

department of Pharmacology, University of Port Harcourt animal house to acclimatize. Fourteen (14) cages covered with normal cage wire mesh in three folds were used for this experiment.

### 2.2 GASOLINE AND DIESEL

The gasoline and diesel used in this experiment were obtained from Mobile filling station, Rumuokwurusi, 2Port Harcourt. These products stored in well-sealed 20 litre plastic containers, were not exposed to light to prevent escape of volatile component and reaction with light.

### 2.3 EXPERIMENTATION

A best ® kitchen scale (moved BS, 2500) was used to weigh the rats and randomly put into 5 groups each for diesel toxicity test, gasoline toxicity test, gasoline pretreatment. The average weight of the rats was 185±0.5g. Increasing volumes of gasoline was soaked in cotton wool and dropped in separate cages for the rats. The mass of the gasoline was determined based on the density of gasoline which is 0.7kg /litre giving the mass as 97.22g /kg. Increasing values of diesel was also soaked in cotton wool and dropped in the cage for the rats to inhale. The density of diesel is 0.84. The mass of the diesel is calculated as 350.0g /kg. The rats were given a half of the $LD_{50}$ of gasoline intraperitoneally (1.18ml) as determined by [18]. They were then exposed to increasing concentration of diesel via inhalation route.

Three control groups were used. The rats in the first control groups were exposed to equivalent volume of normal saline for gasoline by intraperitoneal method. The rats in the second control groups were pretreated with half the $LD_{50}$ of gasoline by intraperitoneal route but not exposed to diesel. The third control group were pretreated with normal saline by intraperitoneal route and exposed to diesel inhalation.A random sample of three (3) animals per group was done and sacrificed about 20 hours later. Alanine transaminase (ALT), aspartate transanimase (AST) and alkaline phosphatase(ALP) were determined from the serum. The animals were dissected and their organs (liver) used for histopathological studies.

## 3.0 RESULTS

### 3.1 EFFECT OF GASOLINE ON BIOCHEMICAL PARAMETERS

Gasoline produced a dose dependent increase in the three liver enzymes.

Table 1: Effect of Gasoline on Liver Enzymes.

| Dose g/kg | ALT (IU) | AST (IU) | ALP (IU) |
|---|---|---|---|
| **Normal gasoline** | 24.0 ± 1.2 | 17.0 ± 1.3 | **58.1 ± 3.0** |
| **49** | 34 ± 1.3 | 29 ± 1.2 | **121.0 ± 2.6** |
| **98** | 47 ± 2.6 | 46 ± 3.0 | **190.0 ± 4.6** |
| **196** | 88 ± 2.4 | 98 ± 2.8 | **189.0 ± 4.8** |
| **392** | **115 ± 3.2** | **126 ± 3.6** | **230.0 ± 2.9** |

n = 3 per sample group mean ± SEM

### 3.2 EFFECT OF DIESEL ON BIOCHEMICAL PARAMETERS.

A dose dependent increase was illicited in the levels of AST, ALT and ALP.

  

Table 2: Effect of Diesel on Liver Enzymes

| Dose g/kg | ALT (IU) | AST (IU) | ALP (IU) |
|---|---|---|---|
| **Normal gasoline** | 24.0 ± 1.2 | 17.0 ± 1.3 | **58.1 ± 3.0** |
| **180** | 30 ± 2.0 | 32 ± 1.0 | **64.2 ± 1.6** |
| **360** | 23 ± 2.5 | 50.4 ± 1.3 | **70.5 ± 3.4** |
| **720** | 32 ± 2.8 | 67.1 ± 3.0 | **82.2 ± 1.3** |
| **1440** | **485.0 ± 1.3** | **76.5 ± 2.4** | **98.4 ± 2.4** |

n = n per sample group mean ± SEM.

### 3.3 EFFECT OF GASOLINE PRE-TREATMENT ON BIOCHEMICAL PARAMETERS.

The increase in the level of AST, ALT and ALP was due to the treatment. This increase when compared to the gasoline curly and the diesel only groups was found to be statistically significant at 5% level of probability using the student t-test

Table 3: Effect of Gasoline Pre-Treatment on Liver Enzymes.

| **Dose g/kg** | **ALT (IU)** | **AST (IU)** | **ALP (IU)** |
|---|---|---|---|
| **Gasoline** | 31.4 ± 1.0 | 35.0 ± 2.0 | **66.2 ± 1.8** |
| **90** | 48.0 ± 1.9 | 39.2 ± 1.3 | **88.2 ± 1.6** |
| **180** | 79.0 ± 3.6 | 69.5 ± 1.5 | **138.5 ± 3.6** |
| **360** | 94.2 ± 2.4 | 100 ± 3.76 | **167.4 ± 3.6** |
| **720** | **118.0 ± 1.2** | **148.5 ± 2.8** | **226 ± 4.1** |

n = 3 per sample group mean ± SEM.

### 3.4 HISTOPATHOLOGICAL EXAMINATIONS

In order to ascertain the level of agreement between biochemical and haematological changes and that of tissues, histopathological studies of the liver, kidney, small intestine and testis were carried out.

**LIVER**

The normal liver architecture was lost in all three groups. The portal tract were congested and enlarged with infarction of hepatocytes patchy and microvascular fatty changes. The group pre-treated with gasoline before diesel showed more marked changes. The control group showed no histopathological changes.

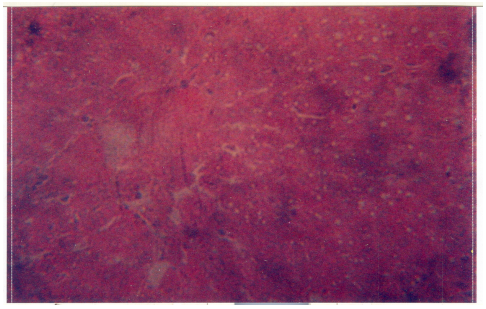

Fig. 1: RAT LIVER (Diesel only) X 100

Showing fatty degeneration.

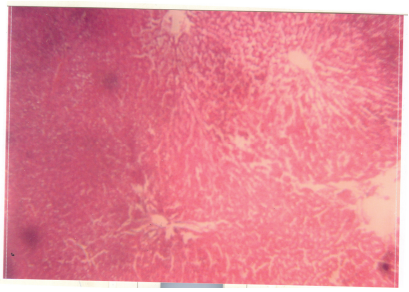

Fig. 2: RAT LIVER (0.9% Saline Pretreatment & Diesel) X 100

Showing dilated sinuses and central vein.

  

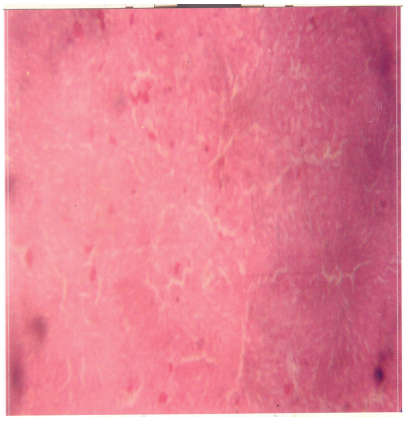

Fig. 3: RAT LIVER (Gasoline Pretreatment & Diesel) X 100

showing necrotic tissues and poorly difined hepatic plates.

**4.0 Discussion**

Liver enzymology was explored to access the biochemical toxicity effects of the samples. Marked changes in liver enzyme level were noted in all the groups except the control group. The increase in aspartate amino transferase (AST), alanine aminotranferase (ALT) and alkaline phosphatase (ALP levels were a consequence of toxic insults on hepatocytes. Diesel is a hepatotoxin considering its ability to cause liver injury after a relatively short time [19]. The extent of liver necrosis and biliary tract function impairment may not be reflected in absolute terms in the level of liver enzymes [20;21;22]. The bilirubin level can also signal hepatocellular damage as demonstrated in guinea pigs exposed to crude petroleum [23]. The presence of gasoline may have increased the absorption of diesel leading to a more lethal effect. The pathology seen in kidney, liver, lungs was as a result of acute toxicity effects of the samples. The liver showed extensive changes in its architecture as it is the seat of metabolism of xenobiotics[14]. The severity of damage in liver cells was in this descending order, gasoline pretreated diesel group, gasoline only group and diesel only group. The control groups showed no significant changes. Patchy hepatocellular necrosis with sinusoidal compression in rats have been shown to result from gasoline poisoning [18].

**5.0 Conclusion**

The findings following this study have shown that interaction of the gasoline and diesel can increase toxicity effects in mammals. The interaction of gasoline and diesel increase the liver enzymes in a dose dependent pattern and cause organ damage as seen in the histomorphology of the livers examined in this study.

**Reference**

[1]. Khana, P.; Devagan, S. C.; Arora, V. K. and Shah, A. (2004) Hydrocarbon Pneumonitis following diesel siphonage. Indian J Chest Dis Allied Sci 46, 129 – 32.

[2]. Obianime, A. W.; Akhidue, V. and Ekanem, N. E. (2001) The effects of inhalational, oral and intraperitoneal administration of gasoline on haematologyical and biochemical parameters of albino rats. Pro.XXII Scientific conference, Physiological Soc. Of Nigeria, Ibadan: 6.

[3]. Koschier, F. J. (1999) Toxicity of middle distillates from dermal exposure. Drug ChemToxicol.n 22, 155 – 64.

[4]. Kuhnhold, W. W. (1980) Some of the impact of aquatic oil pollution of fishery resources FAO/UNDP South Chaina Sea Fishery Development and Co-ordinatingprogramme, Manilla, Philipines Pp. 1 – 26.

[5]. Dede, E. B. and Igbigbi, P. S. (1997) Determination of LD50 value of Metakelfin(R) in rats using Arithemetic method of Karber, J. PhysiolMetascience 4 (1): 10-14 Pp16.

[6]. Periago, J. F. and Prado, C. (2005) Evaluation of occupational exposure to environmental level of aroumatic hydrocarbons in service stations Ann occupHyg 49, 233 – 40.

[7]. International Agency for the Research on Cancer (IARC) (1989) Occupational exposures in petroleum refining; Crude oil and major petroleum fuel. IARC Monographs on the evaluation of carcinoghic risks of humans; Lyon.

  

[8]. Kagbo, Hope D. (1999) Effects of Diesel fuel in the rats (Ratus rats) and tilapla (Oreochromisniloticus)

[9]. CONCAWE (1996) Gas oils (diesel fuels /heating oils). Report Number 95/107.

[10]. Risher, J. F. and Rhodes, S. W. (1995) Toxicological profiles for fuel oils. US Department of Health and Human Sciences.

[11]. International Programme on Chemical Safety (IPCS) (1996) Environmental Health Criteria 171. diesel fuel and exhaust. World Health Organization. Geneva.

[12]. Mayers, C. W. and Jones R. S. (1990) Textbook of liver and Billary Surgery. J. B. Lippin Colt. Company, Philadelphia.Pp 47 - 61

[13]. Wilkinson, H.J. (1976) Diagnostic Enzymology, Edward Arnold Publishers Ltd. London. Pp 527 – 533.

[14]. Kaplowitz, N. (1992) Liver and biliary disease. Williams and Wilkins, London. Pp 3 – 17.

[15]. Ritchie, G. D.; Still, K. R.; Alexander, W. K.; Nordholm, A. F.; Wilson, C. L>; Rossi, J.; Bob and Mattie, D. R (2001) A view of the neurotoxicity risk of selected hydrocarbon fields. J toxicol Environ Health B Crit Rev. 4, 233 – 312.

[16]. Fiedler, N.; Giardion, N. Natelson, B. Ottenweller, J. E.; Weisel, C. Lioy, P.; Lehrer, P.; Ohman-Strickland, P.; Kelly-McNeil, K. and Kipen, H. (2004) Responses to controlled diesel vapor exposure among chemically sensitive Gulf War ventures. Psychosom Med 66, 588 – 98.

[17]. ESSDT (European Society for the Study of Drug Toxicity) (1972) Toxicological Problems of Drugs Combination. ExieptaMedica 13: 790 – 795.

[18]. Igbo, N. M. (1999) Effects of petroleum samples on heamatological and some biochemical parameters of Clariageriepinus, Ratusratus and Celosia argenti. PhD Thesis, Depatment of Biochemistry University of Port Harcourt.

[19]. Jeffries, G. H. (1979) Toxic and Drug-induced liver disease In; Ceal Textbook of Medical Beeson P. B.; MacDermott W.; Wyngarreden, J. B. (eds) 15th Ed., W. B. Saunders, Philadelphia P. 1657 – 1659.

[20]. Greenberger, N. J. (1982) Gastrointestinal disorders; A pathophysiological approach 2nd Ed, Year B medical Pub; Chicago, P 279 – 293.

[21]. Kaplan, M. M. (1990) Evaluation of Hepatobiliary diseases. In : Internal Medicine, Stein, J. H. (eds) 3rd, Ed, Little Brown and co. inc.: Boston P 441 – 443.

[22]. Podolsky, D. K. and Isselbacher, K. J. (1991) Diagnostic tests in liver disease. In: Harrison's Principle of Internal Medicine, Wilson J. D. Braunwald E, Isselbacher K J, Petersdorf R. G., Martin J. B. Favci A. S. and Root R. K. (eds) 12th Ed, McGraw Hill Inc. New York, 2: 1308 – 1309.

[23]. Dede, E. B. and Didia, B. C. (2003) The effects of crude petroleum on bilirubin (diret and total) level of guinea pig JNES 1 (2) P 257 – 261.

  