BIOCHEMICAL EFFECTS OF DIESEL ON SERUM LIPID PROFILE OF ALBINO RATS

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ABSTRACT

The sub-chronic toxicological effect of diesel fuel in rats using some biochemical parameters was investigated. Twenty four (24) rats were divided into four (4) groups with six (6) rats per group. After 7 days of acclimatization, three doses (0.1%, 0.5% and 1.0% /body weights respectively) of diesel fuel were administered into the rat's feed and the effect monitored for the next 2 weeks. HDL-cholesterol, LDL-cholesterol, triacylglycerol and total cholesterol were used as diagnostic markers to ascertain liver dysfunction. The results obtained indicated a non-significant changes (P>0.05) in the HDL-cholesterol and LDL-cholesterol in weeks 1 and 2. There was a significant difference (P<0.05) in the triacylglycerol level of the test groups in weeks 1 and 2. The Serum Cholesterol level showed a significant decrease (P<0.05) in the high percentage (1.0%/kg body weights) compared to low percentage (0.10%) in week 1.

Keywords: Diesel, High density lipoprotein, Low density lipoprotein, Triacylglycerol, Total cholesterol

INTRODUCTION

Normal physiological state of an organism has been found to be altered by so many environmental factors. These factors exist in gaseous, liquid, semi-solid, and solid states which can easily enter into organisms through inhalation, ingestion, dermal contact or diffusion. These substances are termed as xenobiotics and when they get into the organisms they alter the normal state of the system as a result of unwanted chemical reaction they elicit. Therefore, biochemical studies of animals, man precisely, are of ecological and physiological interest. The result of such studies will help in proper understanding of the relationship of biochemical characteristics to the habitat and its adaptability to the environment. Diesel is among these xenobiotics. Diesel is a complex mixture of hydrocarbons produced by mixing fractions obtained from the distillation of crude oil with brand-specific additives to improve performance.

The signs of toxicity following oral intake are generally stated to include nausea, vomiting, diarrhoea, irritation of the aero-digestive and gastrointestinal tracts (CONCAWE, 1996). In one reported case of intentional self-poisoning, chemical pneumonitis was observed (which may have been due to aspiration of vomitus) (Boudet *et al.,* 1983).

Under normal conditions of storage, handling or use as fuel, diesel should not present a hazard to health provided excessive skin contact is avoided (CONCAWE, 1985). 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 (Risher and Rhodes, 1995; IPCS, 1996).

There are few studies investigating the toxicity of diesel per se. Therefore, toxicological evaluations of diesel tend to be derived by considering the toxicity of similar (middle distillate) products such as kerosene and petrol. 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.

Studies on the effect of diesel fuel to man is of vital importance. This is because of the dependency of the normal physiological state of an individual to the biochemical contents/ratios of the body system. The objective of this study was to determine the effects of diesel fuel on the basic lipid profile using rat model.

MATERIALS AND METHODS

Test Sample: Diesel was obtained from the Nigerian National Petroleum Corporation (NNPC) Mega Station along Emene Expressway, Enugu, Nigeria.

Experimental Animals: Twenty four albino rats were obtained from Faculty of Veterinary Medicine, University of Nigeria Nsukka and housed at Home Science, Nutrition and Dietetics Department animal's

house. The rats were divided into four groups, A, B, C, and D of six rats each and acclimatized for a period of ten days. At the end of the acclimatization period the rats were fed with contaminated diet. Group A, B, C, and D were fed with growers mash chicken feed contaminated with 0.00%, 0.10%, 0.50%, and 1.00% w/w respectively. Group A rats served as the controls and were fed with feed with control diet (the same growers mash chicken feed but no contamination). In addition to that, the control group was isolated to avoid inhalation of the crude oil vapour within the vicinity of the other groups. The feeding lasted for a period of twenty one (21) days while the rats were being monitored day by day and results noted. Thereafter, blood samples were collected at Days 14 and 21 day for analysis and labeled accordingly.

Experimental Design: Twenty four (24) female Wistar albino rats were housed in separate cages, acclimatized for seven days and hen divided into four groups of six rat each. The route of administration (exposure) was vice per-os (P/O).

Group 1 was the control group and was fed with the normal rat diet (vital feed) and drinking water

Group II was feed with the rat diet contaminated with 0.1% diesel (w/w) and water ad libitum.

Group III was fed with the rat diet contaminated with 0.5% diesel (w/w) and water ad libitum.

Group IV was fed with the rat diet contaminated with 1% diesel (w/w) and water ad libitum.

The rats were weighed at the beginning of the experiment in order to predetermine the percentage exposure. The period of exposure (feeding with contaminated diet) was twenty one days in all. The first analysis was carried out after the first fourteen days while the second analysis was after the twenty first day of exposure. Several parameters were assayed using the serum of the experimental animals (rats) from the various groups.

Collection of Sample: Blood samples were collected from the rats on the Days 14 and 21 through the median cantus of the rats with capillary tube and immediately transferred into sterile ethylene diamine tetra-acetic acid (EDTA). The samples collected were centrifuged and serum collected for the analysis.

Determination of Serum High Density Lipoprotein (HDL): Dextran sulphate-Mg (II) method for the in vitro determination of HDL-cholesterol in serum, using a Quimica Clinica Applicada (QCA) test kit. (Quimica Clinica Applicada, Spain). Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were precipitated from serum by the action of a polysaccharide, in the presence of divalent cations. Then, high density lipoproteins cholesterol (HDL-Cholesterol) present in the supernatant is determined (Albers *et al.,* 1978; Benzie, 1979; Wieland and Seidel, 1981).

Determination of Serum Low Density Lipoprotein (LDL): Polyvinyl sulphate method for the in-vitro determination of LDL-cholesterol in serum, using a Quimica Clinica Applicada (QCA) test kit. (Quimica Clinica Applicada, Spain). LDL-Cholesterol can be determine as the difference between total Cholesterol content of the supernatant after precipitation of the LDL fraction by Polyvinyl sulphate (PVS) in the presence of Polyethylene glycol monomethyl ether (Assmann *et al.,* 1984; Demacker *et al.,* 1984).

Determination of Serum Triacylglycerol: The glycerol-phosphate oxidase method (enzymatic test) for the in vitro determination of triacyglycerol in serum, using a Quimica Clinica Applicada (QCA) test kit (Quimica Clinica Applicada, Spain).

Determination of Serum Cholesterol: Enzymatic colorimetric test (CHOD-PAP method) for the in vitro determination of cholesterol in serum or plasma, using Quimica Clinica Applicanda (QCA) and Cholesterol test kit (QCA, Spain).

Statistical Analysis: One way ANOVA statistical analysis was performed to evaluate significant levels between the different groups of experimental rats. The data were expressed as mean ± SD. The level of significance was considered at 95% confidence level.

RESULTS AND DISCUSSION

Effect of Diesel on High-Density Lipoprotein (HDL) Levels: Non-significant difference ($P>0.05$) in the HDL level of the control group and the test groups administered different percentages (0.10, 0.05 and 1.0% /kg body weights respectively) of diesel in Weeks 1 and 2 was recorded. Also, there was non-significant difference ($P>0.05$) between the HDL level of the test groups administered (per-os) the low percentage (0.10%/kg body weight) of diesel when compared to higher percentages (0.50% and 1.0%) of contamination in the weeks 1 and 2 (Figure 1).

Effect of Diesel in Low-Density Lipoprotein (LDL) Level: Non-significant difference ($P>0.05$)

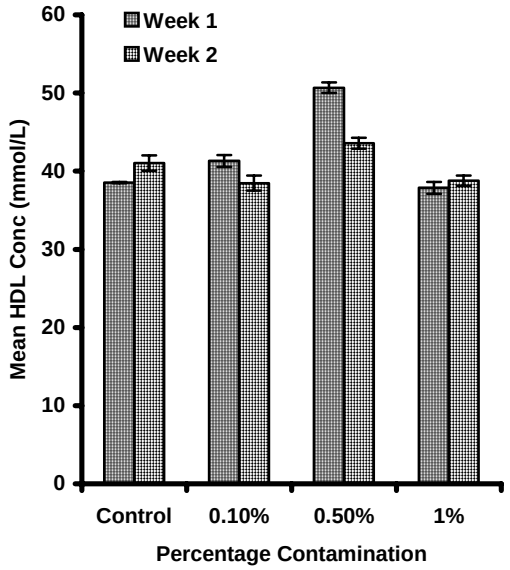

Figure 1: Effect of Crude oil on high density lipoprotein (HDL) concentration

was observed between the LDL level of the control group and that of the various test groups (0.10, 0.50 and 1.0%/kg body weights respectively) administered diesel contamination in week 1. However, in Weeks 2, significant difference ($p<0.05$) was observed in the LDL level of the test groups (0.10, 0.50 and 1.0%/kg body weights respectively) administered diesel. There was non-significant difference ($P>0.05$) across the test groups (0.10, 0.50 and 1.0%/kg body weights respectively) in week 1, while week 2 showed a significant difference ($P<0.05$) in the LDL level of the test groups administered diesel contamination (Figure 2).

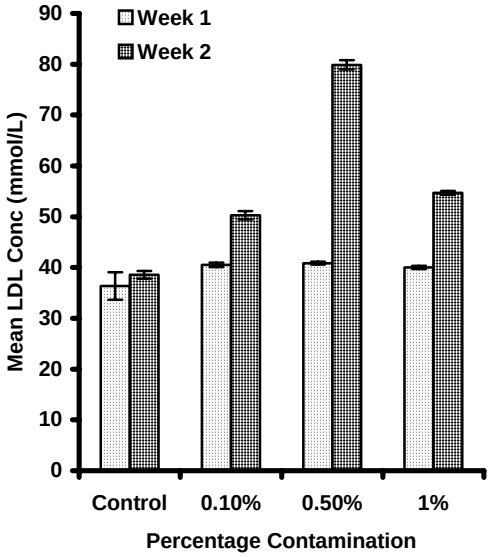

Figure 2: Effect of Crude oil on low density lipoprotein (LDL) concentration

Effect of Diesel on Triacylglycerol (TAG) Level: Figure 3 showed non-significant difference ($P>0.05$) between week 1 of the control group and week 1 of test groups administered low percentage (0.10%/kg body weight) contamination. Significant difference ($P<0.05$) existed when week 1 of the control was compared with week 1 of both the high percentages (0.05 and 1.0%/kg body weights respectively) of contamination. There was non-significant difference ($P>0.05$) across the test groups of week 1. Furthermore, the result at Week 2 revealed non-significant difference ($P>0.05$) when compared to the control group and the highest (1.0%/kg body weight) contamination group. However, 0.1% and 0.50% contamination groups were found to be significantly different ($P<0.05$) when compared to the control group.

Effect of Diesel on Total Cholesterol Level: It was observed that significant difference ($P>0.05$) existed in the total cholesterol level between the control group and test groups (0.10 and 0.50 %/'kg body weights respectively) at Week 1. However, significant difference ($P < 0.05$) existed between the high percentage test group (1.0%/kg body weight) when compared to the low percentage (0.10%/kg body weight) test group. But 0.50% was found to have non-significant difference ($P > 0.05$) with reference to low percentage (0.10%/kg body weight) contamination. There was non-significant difference ($P>0.05$) between the control group and the test groups (0.10, 0.50 and 1.0%/kg body weights respectively) at Week 2. Also, comparison of the week 2 different test groups showed non-significant difference ($P > 0.05$) in the total cholesterol level (Figure 4).

The consumption of diesel contaminated feed may manifest in various forms including changes in biochemical parameters. In the present study, evidences are presented to address the possible effects that can emanate from oral exposure to diesel fuel. The result of the experiment carried out showed that changes that occurred in weeks 1 and 2 of both high density lipoprotein (HDL) and low density lipoproteins (LDL) level respectively of the test groups were not significant ($P>0.05$) when compared to their control groups. This suggested that diesel fuel apparently does not have an adverse effect on both HDL and LDL levels of albino rat. Week 2 of TAG result shows a significant increase ($P<0.05$) when compared to TAG levels in week 1. However, in week 1, significant decrease ($P<0.05$) was observed across the test groups with higher percentages (0.5% and 1.0%) when compared to the control group of the same week 1.

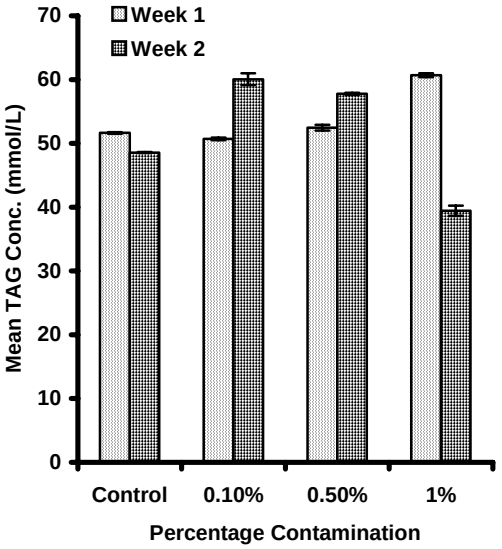

Figure 3: Effect of Crude oil on triacyglycerol (TAG) concentration

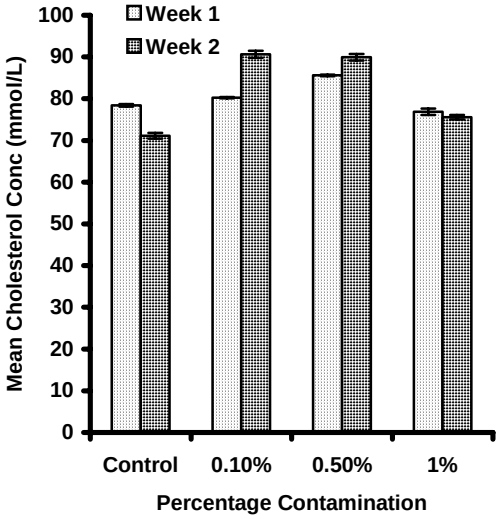

Figure 4: Effect of Crude oil on total cholesterol concentration

The total cholesterol level was found to be opposite of that gotten from TAG. Total cholesterol was found to have a significant decrease ($P<0.05$) in week 2 compared to week 1 total cholesterol level. These alterations in TAG and total cholesterol levels in weeks 1 and 2 suggest that adverse effect occurred on the liver.

Lipid metabolism is affected once there is liver damage since the disturbance of cell membrane integrity is likely to cause some membrane lipids to be released into circulation; while on the other hand, it causes the tissue to compromise its effectiveness in regulating lipid metabolism (Murray *et al.,* 2003). Each class of lipoprotein has a specific function, determined by its point of synthesis, lipid composition, and apolipoprotein content distinguishable by their size, their reactions with specific antibodies, and their characteristic distribution in the lipoprotein classes (Murray *et al.,* 2003). In mammals, cholesterol production is regulated by intracellular cholesterol concentration and by the hormones glucagon and insulin. The rate-limiting step in the pathway to cholesterol (and a major site of regulation) is the conversion of HMG-CoA to mevalonate, the reaction catalysed by HMG-CoA reductase. To activate transcription of the HMG-CoA reductase gene and other genes, the transcriptionally active domain is separated from the rest of the sterol regulatory element-binding protein (SREBP) by proteolytic cleavage. The enzyme exists in phosphorylated (inactive) and dephosphorylated (active) forms. Glucagon stimulates phosphorylation (inactivation), and insulin promotes dephosphorylation, activating the enzyme and favoring cholesterol synthesis (Murray *et al.,* 2003).

Unregulated cholesterol production can lead to serious human disease. When the sum of cholesterol synthesized and cholesterol obtained in the diet exceeds the amount required for the synthesis of membranes, bile salts, and steroids, pathological accumulation of cholesterol in blood vessels (atherosclerotic plaques) can develop, resulting in obstruction of blood vessels (atherosclerosis). Atherosclerosis is linked to high levels of cholesterol in the blood, and particularly to high levels of LDL-bound cholesterol; there is a negative correlation between HDL levels and arterial disease (Murray *et al.,* 2003).

Previous studies revealed that some individuals may experiment adverse effects to diesel fuels due to genetic polymorphisms in their ability to biotransform xenobiotics and solvents (Ritchie *et al.,* 2001). The imbalance in phase I drug metabolizing enzymes and phase II drug detoxification enzymes will lead to increased levels of bioactivated compounds and impaired detoxification leading to potential adverse health effects (Ritchie, 2003). Increased risk for prostrate cancer and squamous cell carcinoma in the lungs has been reported in a multi-site, case control study (Siemiatycki *et al.,* 1987). However, it was conceded that these effects could have resulted from any particular chemical. There is no clear evidence that diesel fuels are genotoxic. Hence, cancer could be induced by nongenotoxic mechanisms, such as chronic dermal irritation characterized by repeated cycles of skin lesions, causing epidermal hyperplasia.

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