



PROTECTIVE EFFECT OF VITAMINS A AND E AGAINST DIESEL FUEL-INDUCED HEPATOTOXICITY IN RATS

PRESENTED BY

FRIDAY E. UBOH, *PhD, MIPAN*

**BIOCHEMISTRY DEPARTMENT
FACULTY OF BASIC MEDICAL SCIENCES
UNIVERSITY OF CALABAR
CALABAR - NIGERIA**

E-mail: fridayuboh@unical.edu.ng & fridayuboh@yahoo.com



INTRODUCTION:

- Diesel fuel is one of the fractions derived from the fractionation of crude oil. It has a mixture of different types of hydrocarbons as the major chemical constituents.
- According to Speight (1992); ATSDR (1995), the primary hydrocarbon in diesel fuel are those with 12-20 carbon atoms per molecule; comprising 45% cycloalkanes, 30% n-paraffin and about 25% aromatic hydrocarbons.

- The use of diesel fuel in automobile and light generating engines in industrial and domestic environments is very common in many developing societies worldwide.
- Hydrocarbons (including those from diesel fuel) are very volatile, and constitute ubiquitous chemical pollutants in domestic and workplace environment environments where diesel fuel is dispensed or used.

- Exposure to these hydrocarbons is therefore common at various environments where diesel fuel is dispensed or used.
- Some aromatic and aliphatic hydrocarbon are normally metabolized to reactive species, which interact with the endogenous macromolecules to produce oxidative stress inducing species (Hu et al., 1994; Ueng et al., 1998; MohanKumar et al., 2008; Lin et al., 2009; Van Scoy et al., 2010).

- Previous studies showed that exposure to petroleum fractions resulted in oxidative stress induced hepatotoxicity (Dede and Kagbo, 2001; Uboh et al., 2007, 2009; Milinkovitch et al., 2011; Patrick-Iwuanyanwu et al., 2011).
- Diesel induced toxicities have been demonstrated with the evidenced increase in tissue MDA, ALT, AST, as well as decrease in GSH, and SOD (Uboh et al., 2007, 2009, 2013; Milinkovitch et al., 2011)

Introduction Cont'd

- The use of petroleum based fuels in most developing societies is very common.
- Hence, the toxicity effects reported to be associated with exposure to petroleum hydrocarbons therefore become a major concern of the health related scientific societies.
- Literature reports that different chemical substances may be used to ameliorate the reported petroleum hydrocarbons induced toxicities.

Introduction cont'd

- In our previous studies, vitamins A, C and E have been demonstrated to provide protection against gasoline vapour induced hepatotoxicity in rats (Uboh et al., 2009b, 2012). Also, the use of indigenous plants to ameliorate petroleum products and carbon tetrachloride intoxication has been reported (Kalu et al., 2011; Ujowundu et al., 2012).
- In this study, we assessed the protective effects of vitamins A and E on diesel fuel induced hepatotoxicity in rats.

MATERIALS AND METHODS:

- **Animal Handling and Treatment:** Forty two apparently normal matured male albino Wistar rats (180 to 200g), obtained from Biochemistry Department Experimental Research Animal House of the University of Calabar, Calabar, Nigeria, were used in this study. They were fed with standard laboratory diet and tap water. The work was carried out under 12 hours light/dark cycle illumination and room temperature of $25 \pm 2^{\circ}\text{C}$.
- The animals were distributed into five groups, with seven rats each, as shown below:

Materials and Methods Cont'd

- **Group 1:** Received 1.0ml/kg body weight of distilled water for 30 days
- **Group 2:** Received 1.0ml/kg body weight of vegetable oil for 30 days
- **Group 3:** Received 4.0ml/kg body weight of diesel fuel for 30 days.
- **Group 4:** Received 4.0ml/kg body weight of diesel fuel + 400IU/kg body weight of vitamin A for 30 days
- **Group 5:** Received 4.0ml/kg body weight of diesel fuel + 200IU/kg body weight of vitamin E for 30 days
- **Group 6:** Received 4.0ml/kg body weight of diesel fuel + 200IU and 400IU/kg body weight of vitamins E and A, respectively, for 30 days

Materials & Methods Cont'd

- The animals were sacrificed, 24 hours after the 30th day of experimental period.
- The study was carried out according to the Guidelines of the Institution's (University of Calabar, Nigeria) Animal Research Ethics Committee, with reference to the Guide for the Care and Use of Laboratory Animals (NRC, 1995).

- Collection and preparation of blood and liver tissues for analyses: Blood samples were obtained from rats by cardiac puncture, under chloroform vapor anaesthesia, 24 hours after termination of experimental exposure, into sterile plain sample bottles for biochemical indices.
- The liver tissues were also collected for tissue oxidative stress bioindicators.

- The blood samples collected for biochemical indices assay were allowed to clot and centrifuged with Table-top centrifuge (MSE model, England) at 3000 rpm for 10 minutes to obtain the serum, which was subsequently used for the biochemical assay.
- Liver tissues were also collected (immediately perfused in ice cold 0.9% NaCl solution), and used for the estimation of tissue oxidative stress bioindicators.

Biochemical analyses: The activities of serum alanine and aspartate transaminases (ALT and AST), γ -glutamyltransferase (GGT), alkaline phosphatase (ALP), liver tissue superoxide dismutase (SOD), catalase and malondialdehyde (MDA) were determined using Biosystems Laboratories (S. A. Costa Brava, Barcelona, Spain) and Randox Laboratories (United Kingdom) reagent kits.

Materials & Methods Cont'd

- Serum lipid profile was determined using analytical grade reagent kits obtained from Randox Chemical Company, UK. The “high performance” enzymatic colorimetric, CHOD-PAP method described by Richmond was used to assess total serum cholesterol.
- Serum triacylglycerol levels were estimated by the modified enzymatic colorimetric, glycerol phosphate oxidase (GPO), method of Trinder (1969).
- HDL-cholesterol estimation was done using the chylomicrons precipitation method of Richmond (1973).

- VLDL and LDL cholesterols were obtained by calculation using the empirical relationships of Friedwald et al. (1972).
- **Statistical Analysis:** Results were presented as mean \pm S.E.M and statistically analysed using one-way analysis of variance (ANOVA) with SPSS window statistical software programme. Student "t" test was used for pair-wise comparison, and differences were considered significant at $p < 0.05$.

RESULTS

- The results of this study are presented in Tables 1 - 5. The results showed that serum ALT, AST, GGT and ALP activities, liver tissue MDA, total cholesterol, triacylglycerol, LDL and VLDL levels increased by exposure to diesel fuel were significantly ($p < 0.05$) reduced to levels within the control range by vitamins A and E, administered either individually or in combination.
- Moreover, liver SOD activity and serum HDL level decreased by diesel fuel were also significantly ($p < 0.05$) increased to levels within the control range by the vitamins.

Table I. Effect of combined administration of vitamins A and E on serum ALT and AST activities of rats orally exposed to diesel

Group	Treatment	ALT (IU/dl)	AST (IU/dl)
1	Distilled water	16.8±2.4	18.5±2.6
2	Vegetable oil	17.2±2.7	17.8±2.8
3	Diesel fuel only	38.5±3.8*	40.2±4.2*
4	Diesel fuel + Vit. A	22.1±3.3 ^a	23.5±3.1 ^a
5	Diesel fuel + Vit. E	20.6±2.7 ^a	21.6±2.6 ^a
6	Diesel fuel + vits A &E	19.8±2.2 ^a	20,2±2.4 ^a

Values are presented as mean ± SEM; n = 7. *P<0.05 compared with “Grp” 1 and 2; ^aP<0.05 compared with “Grp” 3

Table 2. Effect of combined administration of vitamins A and E on serum GGT and ALP activities of rats orally exposed to diesel

Group	Treatment	GGT (IU/dl)	ALP (IU/dl)
1	Distilled water	2.6±0.6	32.6±4.2
2	Vegetable oil	2.8±0.5	33.9±4.8
3	Diesel fuel only	6.8±1.3*	82.7±5.8*
4	Diesel fuel + Vit. A	3.5±0.6 ^a	40.3±4.7 ^a
5	Diesel fuel + Vit. E	3.3±0.8 ^a	38.1±4.6 ^a
6	Diesel fuel + vits A & E	3.1±0.8 ^a	36.8±4.8 ^a

Values are presented as mean ± SEM; n = 7. *P<0.05 compared with “Grp” 1 and 2; ^aP<0.05 compared with “Grp” 3

Table 3. Effect of combined administration of vitamins A and E on liver tissue SOD, catalase activities and MDA levels of rats exposed to diesel

Group	SOD (U/g tissue)	Catalase (U/g tissue)	MDA (U/g tissue)
1	28.5±3.2	12.3±2.3	2.1±0.3
2	27.8±2.8	10.8±2.1	2.3±0.6
3	15.6±2.6*	5.3±1.8*	8.5±2.6*
4	24.3±3.4 ^a	9.2±2.0 ^a	3.9±0.8 ^a
5	25.6±3.6 ^a	10.3±2.2 ^a	3.2±0.6 ^a
6	26.8±3.5 ^a	11.6±2.6 ^a	2.9±0.4 ^a

Values are presented as mean ± SEM; n = 7. *P<0.05 compared with “Grp” 1 and 2; ^aP<0.05 compared with “Grp” 3

Table 4. Effect of combined administration of vitamins A and E on serum total cholesterol, triacylglycerol and HDL-cholesterol levels of rats exposed to diesel

Group	Total cholesterol (mmol/l)	Triacylglycerol (mmol/l)	HDL-Chol. (mmol/l)
1	2.6±0.7	2.4±0.6	4.6±1.4
2	2.8±0.9	2.6±0.7	4.2±1.1
3	6.5±1.7*	5.8±1.5*	2.1±0.7*
4	3.0±1.1 ^a	2.9±0.7 ^a	3.9±1.0 ^a
5	2.8±0.8 ^a	2.6±0.8 ^a	4.2±1.4 ^a
6	2.7±0.6 ^a	2.5±0.6 ^a	4.4±1.6 ^a

Values are presented as mean ± SEM; n = 7. *P<0.05 compared with “Grp” 1 and 2; ^aP<0.05 compared with “Grp” 3

Table 5. Effect of combined administration of vitamins A and E on serum LDL-cholesterol and VLDL-cholesterol levels of rats exposed to diesel

Group	Treatment	LDL-C (mmol/l)	VLDL-C (mmol/l)
1	Distilled water	1.3±0.4	1.5±0.4
2	Vegetable oil	1.4±0.3	1.6±0.6
3	Diesel fuel only	3.8±1.1*	3.6±1.0*
4	Diesel fuel + Vit. A	2.0±0.8 ^a	1.9±0.6 ^a
5	Diesel fuel + Vit. E	1.8±0.5 ^a	1.7±0.4 ^a
6	Diesel fuel + vits A &E	1.5±0.4 ^a	1.6±0.5 ^a

Values are presented as mean ± SEM; n = 7. *P<0.05 compared with “Grp” 1 and 2; ^aP<0.05 compared with “Grp” 3

CONCLUSION:

- In summary, the results of this study showed that the chemical constituents of diesel fuel introduced into the body during exposure are capable of inducing oxidative stress.
- And that induction of oxidative stress is the mechanism through which exposure to diesel fuel induces hepatotoxicity.
- These results are consistent with our earlier report on the gasoline vapour induced oxidative stress in rats (Uboh et al., 2012, 2013).

Conclusion Cont'd

- Hepatoprotective potentials of vitamins A and E, administered individually or in combination, against diesel fuel induced hepatotoxicity is also indicated by the results of this study.
- This observation indicated a comparable antioxidative, hence hepatoprotective, properties of vitamins A and E against diesel fuel induced oxidative stress, and hence hepatotoxicity in rats.

Conclusion Cont'd

The result reported in this study is also consistent with the report of our earlier studies on the effect of vitamins A, C and E on gasoline vapour induced hepatotoxicity in rats (Uboh et al., 2011, 2012, 2013).

- It may therefore be concluded that vitamins A and E possess a comparable protective properties against diesel fuel-induced hepatotoxicity in male albino Wistar rat model.

ACKNOWLEDGEMENT

- All my Postgraduate students.
- All the technical staff of Biochemistry Dept., University of Calabar, Nigeria.
- Dr. Godwin Iwatt, in blessed memory, and all the technical staff of Pentamed Laboratories, Calabar, Nigeria.



THANK

YOU

REFERENCES:

- Faber WD, Roberts LSG, Stump DG, Tardif R, Krishnan K, Tort M, Dimond S, Dutton D, Moran E and Lawrence W (2006). Two-generation reproduction study of ethylbenzene by inhalation in Crl-CD rats. *Birth Defects Res. B Dev. Reprod. Toxicol.* 77: 10-21.
- Jain SK and Subrahmanyam D (1978). On the mechanism of phenylhydrazine-induced hemolytic anemia. *Biochemical and Biophysical Research Communications* 82(4) 1320-1324.



References Cont'd

Joshi PK, Bose M, and Harish D (2002). Changes in certain haematological parameters in a siluroid catfish *Clariasbatrachus* (Linn) exposed to cadmium chloride. *Pollution Resources*; 22:129-131.

Jovanovic JM, Jovanovic MM, Spasic MJ, Lukic SR. 2004. Peripheral nerve conduction study in workers exposed to mixtures of organic solvents in paint and lacquer industry. *Croat. Med. J.* 45: 769-774.

References Cont'd

- Lundberg I, Nise G, Hedenborg G, Hogberg M, Vesterberg O (1994). Liver function tests and urinary albumin in house painters with previous heavy exposure to organic solvents. *Occupational Environ. Med.* 51: 347-353.
- Michalopoulos GK, DeFrances MC (1997). Liver regeneration. *Science* 276:60.
- Patil AJ, Bhagwat VR, Patil JA, Dongre NN, Ambekar JG, Das KK (2007). Occupational lead exposure in battery manufacturing workers, silver jewelry workers, and spray painters in Western Maharashtra (India): effect on liver and kidney function. *J. Basic Clin. Physiol. Pharmacol.* 18(2): 87-100.



References Cont'd

Ramirez-Farias C, Madrigal-Santillan E, Gutierrez-Salinas J et al. (2008). Protective effect of some vitamins against the toxic action of ethanol on liver regeneration induced by partial hepatectomy in rats. *WJG* 14: 899-907.

Seeber A, Sietmann B, Zupanic M. (1996). In search of dose-response relationships of solvent mixtures to neurobehavioral effects in paint manufacturing and painters. *Food Chem. Toxicol.* 34: 1113 -1120.

References Cont'd

Uboh FE, Akpanabiatu MI, Alozie Y, Edet EE, Ndem JI and Ebong PE (2009) Comparative effect of vitamins A and E on gasoline vapours-induced haematotoxicity and weight-loss in male Rats. *International Journal of Pharmacology* 5(3): 215-221.

Uboh FE, Akpanabiatu MI, Aquaisua AN and Basse EI (2012a). Oral Exposure to Nitrocellulose Thinner Solvent Induces Nephrotoxicity in Male Albino Wistar Rats. Journal of Pharmacology and Toxicology 7(2): 78-86

References Cont'd

Uboh FE, Usoh IF, Nwankpa P and Obochi GO. (2012b). Effect of Oral Exposure to Nitrocellulose Thinner on Haematological Profiles of Male Albino Wistar Rats. American Journal of Biochemistry and Molecular Biology 2(4): 227-234

World Health Organization (WHO). (2005). Concise International Chemical Assessment Document 64. Butyl Acetates. World Health Organization: Geneva.