Original Research Article

Vitamin C Supplementation Lowered Atherogenic Lipid Parameters Among Oil and Gas Workers Occupationally Exposed to Petroleum Fumes in Port Harcourt, Rivers State

ABSTRACT

Aim: To evaluate the effect of vitamin C supplementation on lipid profile parameters among oil and gas workers occupationally exposed to petroleum fumes through inhalation over a period of 12 months.

Study Design:A total of 150 subjectsbetween the age ranges of 18 to 45 years old were recruited for the study of which 50 subjects were exposed to petroleumfumes and were given vitamin C supplementation (group1), 50were also exposed to petroleum without vitamin C (Group 2) and 50 subjects are non-oil and gas workers (control subjects).

Methodology: The Group 1 subjects were orally administered vitamin C supplements of 100mg/day for 60 days prior to sample collection while Group 2 subjects exposed to petroleum fumes were not given vitamin C or took vitamin during the period of the study. The control subjects were non-oil and gas workers. At the end of the experiment (60 days), blood specimens were collected from group1, group2, and the control subjects. Lipid parameters such as cholesterol, triglycerides, High-Density lipoprotein, Low-Density lipoprotein, and very low-density lipoprotein were analysed using enzymatic methods. Statistical analysis of raw data was done using SPSS and results were expressed as Mean±Standard Deviations.

Results: The results indicated significantly higher values in total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, and very low density lipoprotein in the petroleum fumes exposed subjects compared to control subjects. When those exposed and given vitamin C supplementation were compared with those exposed without vitamin C, the results indicated significantly higher values in subjects exposed to petroleum fumes without Vitamin C supplementation compared to the subjects exposed to petroleum fumes given 100mg of Vitamin C supplementation for 60 days. It was further observed that there were significantly higher values in the lipid parameters considered in workers exposed to petroleum fumes compared with control subjects at P=.05. However, no significant difference was observed between the control subjects and those exposed to vitamin C supplementationat P=.05.

Conclusion: The study has shown that occupational exposure to petroleum fumes is associated with dyslipidaemia. However, the use of 100mg of vitamin C daily for 60 days ameliorated the degree of dyslipidaemia associated with occupational exposure to petroleum fumes. Vitamin C could be considered as a preventive means to mitigate or ameliorate the adverse effects of occupational exposure to petroleum fumes and products among workers.

Keywords: Gasoline pump workers, gasoline, lipid profile, vitamin C, anti-oxidant, petroleum fumes, Dyslipidaemia, cardiovascular risk

1. INTRODUCTION

Lipids function as hormones, energy sources and structural components in cell membranes and are transported in the plasma binding to specific carrier proteins called lipoproteins [1].Lipid particles such as total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein,and very-low density lipoprotein are vital biochemical parametersusually employed as a frontline approach in the screening for abnormalities in lipids metabolism viz-a-viz in disease diagnosis such as cardiovascular diseases[2, 3]. Lipoproteins include chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) [2].Abnormalities in the metabolism of lipids and lipoproteins are linked with the risks of developing atherosclerosis, one of the underlying causes of cardiovascular disorders such as myocardial infarction, cerebrovascular diseases and peripheral vascular disease [2, 3]. Several factors such as exposure to drugs, chemicals, and lifestyle have been reported to affect lipid levels. Elekima et al.[1],2 documented that, oral administration of carmoisine(a food dye) affected the lipid metabolism with a resultant increase in cholesterol level. Exposure to petroleum or petroleum fume could also serve as a risk for dyslipidaemia.

Petrol is a clear petroleum-derived flammable liquid that is used primarily as fuel in most spark-ignited internal combustion engines[4]. It consists mostly of organic compounds obtained by the fractional distillation of petroleum, enhanced with a variety of additives [5].The hazardous effect of petrol (fuel) on human health has greatly been of public health concern and filling station fuel pump workers are an important group who are at occupational risk to BTX (benzene, toluene, and xylene) compounds which are the main constituents of petrol of which Benzene stands out for its hazardous effects on human health[4, 6, 7]. Keenan et al. [8], documented that that simultaneous exposure to benzene and other aromatic hydrocarbons, such as toluene and xylene, contributes to maximizing benzene toxicity and oxidative stress.

There are health concerns for workers occupationally exposed to benzene [4, 5]. These concerns are linked to the fact that benzene is a well-recognized genotoxic human carcinogen, classified as a Group I chemical by the International Agency for Research on Cancer, and without any known threshold dose [7].Occupational Safety and Health Administration [5] has set a permissible exposure limit of 1 ppm of benzene in airat the workplace during an 8-hour workday, and 40-hour workweek. OSHA, [5], further stated that the short-term exposure limit for airborne benzene is 5 ppm for 15 minutes. The U.S. National Institute for Occupational Safety and Health (NIOSH) in 2004 revised the immediately dangerous to Life and Health (IDLH) concentration for benzene to 500ppm.

Badham et al.[9], documented that the activation of benzene and its metabolites in petroleum products culminates in damage to lipids, proteins, and DNA, & carbohydrates through various chemical reactions involving oxidative stress, leading to functional alterations in different tissues. Moro *et al.,*[4] also further reported that exposure to benzene in petroleum productsexposure can lead to non-cancer health effects, such as genotoxicity, hematotoxicity, hepatotoxicity, and nephrotoxicity. The mechanism of action of their toxicity has been linked with the rapid generation of free radicals overwhelming the anti-oxidant capacity of the system leading to peroxidation of lipids and consequently inducing local injury to cell membranes[10, 11].

Free radicals are reactive chemicals that possess the capacity to disrupt cellular activities. These radicals are usually generated during oxidative processes when an atomic or molecular moietyeither gains or loses an electron. They could be introduced into the system internally through metabolic processes or externally [12]. Free radicals in required physiological levels in the blood and cellular components are involved in cellular signals and other vital functions [2, 12]. However, when their production overwhelms the anti-oxidant capacity, it becomes pathologic resulting in different disorders affecting the macromolecules and micro-molecules, including DNA, proteins, lipids, proteins, and cell membranes.Free radicals with oxygen as the active agent are referred to as reactive oxygen species (ROS) while those with nitrogen are referred to as reactive nitrogen species (RNS). ROS are the most common type of free radicals produced in living tissue[12]]. Theeffects of these free radicals are usually counteracted by an antioxidant defense mechanism.

Antioxidants are chemicals whose main function is in the eradication of free radicals, allowing physiologically permissible levels [12]. Antioxidants are produced within the system (endogenous antioxidants) or derived (Exogenous anti-oxidants). Irrespective of the endogenous anti-oxidant generation, the biological system also depends on exogenous sources, mainly, through the diet (fruits, vegetables, and grains) and dietary supplements[12]. Examples of dietary antioxidants include beta-carotene, lycopene, and vitamins A, C, and E. The human body'scomplex antioxidant defense system constitutes dietary intakes of antioxidants in the form of fruits and vegetables and endogenous production of antioxidant compounds e.g. glutathione [12].

Vitamin C (Ascorbic Acid) is considered a vitamin with a significant anti-oxidative effect and by extension could be exceptionally gainful in the therapy of oxidative-induced organ damage. Studies by George-Opuda et al., [13],revealed that Vitamin C reduced theimpact of gasoline exposure in rats. Vitamin C is a powerful reducing agent that is water soluble vitamin required for the maintenance of skin and involved in the removal of reactive species. The essence of the study is to assess the influence of Vitamin C on the lipid parameters after exposure to petroleum fumes.

2. Materials and Methods

2.1 Materials

The materials used in this study included vitamin C, a vacutainer, lithium heparin bottles, an automatic pipette, test tubes, and a spectrophotometer.

2.2 Study Design

A total of 150 healthy adults between the ages of 18 to 45 years were recruited for the study.Hunderdof the participants are working as fuel attendants at gas stationsexposured to petroleum fumes. Of these 100 exposed subjects, 50 subjects (Group1) were given 100mg of vitamin C supplements daily for 60 days while the other 50 (Group 2) were not given vitamin C or took vitamins during the study period. The control subjects were non-oil and gas workers. After 60 days, blood samples were collected from the control group, and exposed groups for laboratory analyses after 12 hours fast.

2.3 Subjects Selection Criteria

2.3.1 Inclusion Criteria

Subjects included in the study were between the ages of 18-45 years. They are non-alcoholics, not on any medication, or chronic diseases. Also, the filling station workers had worked in the fuel station for a minimum of one year.

2.3.2 Exclusion Criteria

Those excluded included alcoholics, those with a history of cardiac or cardiac-related disease, and those on medication. Staff members recently employed or have worked less than 12 months were also excluded.

2.4 Informed Consent

Informed consent was obtained from the subjects before specimens were collected upon clearance from the Department of Medical Laboratory Science, Rivers State University.

2.5 Sample Collection and Preparation

Venous blood specimens in fasting states werecollected into lithium heparin bottles and centrifuged to obtain plasma. The plasma obtained was used to determine the concentration of cholesterol, triglycerides, and high-density lipoprotein.

2.6 Sample Analysis

Cholesterol and triglycerides were determined using the enzymatic methods.High-density lipoprotein (HDL) cholesterol wereanalysed using the precipitation method while LDL cholesterol levels was calculated using Friedwald's equation as described by Friedwald et al. [14].

Statistical Analysis

Statistical analysis was performed using SPSS. Results were presented as Mean±SD. Students't-test was used to compare between control and test subjects. Also, One-Way ANOVA (Post Hoc: Tukey's multiple comparative tests) was used to determine the significantdifferences between Group1, Group 2, and the controls.

3 RESULTS

3.1 Result of Lipid Parameters in Subjects Exposed to Petroleum Fumes

The results indicated significantly higher values in total cholesterol, triglyceride, high-density lipoprotein,low-density lipoprotein, and very low-density lipoprotein in the fuel-pump exposedsubjects at *P*=.05 (Table 1).

Formatted: Underline

Table 1.Results of Lipid Parameters in Subjects Exposed to Petroleum fumes

Parameters	Exposed (Test)	Unexposed (Control)	P value	Remark
TCHOL(mmol/L)	4.629±0.449	3.980±0.555	<0.0001	S
TRIG (mmol/L)	1.196±0.301	0.908±0.505	0.0008	S
HDL (mmol/L)	1.034±0.211	0.936±0.181	0.0155	S
LDL (mmol/L)	3.002±0.509	2.635±0.500	0.0004	S
VLDL (mmol/L)	0.520±0.154	0.408±0.232	0.0057	S

Keys: HDL= High-density lipoprotein, TCHOL = Total cholesterol, LDL= Low-density lipoprotein, VLDL= Very low-density lipoprotein. S=Significant at *P*=.05

3.2 Result of Lipid Parameters in Control Subjects, Petroleum Fume Exposed-Vitamin C supplementation, and Petroleum fume Exposed without Vitamin C rewrite the sentence

The results indicated significantly higher values in subjects exposed to petroleum fumes without Vitamin C supplementation compared to the subjects exposed to petroleum fumes given 100mg of Vitamin C supplementation for ~~30~~ 60?days. It was further observed that there were significant differences in the values of the lipid parameters considered when workers exposed to petroleum fumes were compared with control subjects at *P*=.05 (Table 2).

Table 2: Result of Lipid Parameters in Control Subjects, Petroleum Fume Exposed-vitamin C Supplementation, and Petroleum Fume Exposed without Vitamin C Supplementation

Parameters	Control	Exposed + Vitamin C	Exposed Without	P value	Remark

		(Group 1)	Vitamin C (Group 2)		
T.CHOL(mmol/L)	4.103 ± 0.222[a]	4.201±0.123[a]	4.687 ± 0.841[b]	<0.0001	S
TRIG (mmol/L)	1.004 ± 0.201[a]	1.210±0.658[a]	1.496 ± 0.801[b]	0.0006	S
HDL (mmol/L)	0.9364 ±0.181[a]	0.8947±0.222[a]	1.187 ± 0.194[b]	0.0166	S
LDL (mmol/L)	2.635 ±0.100[a]	2.234±0.231[a]	3.982 ± 0.604[b]	0.0002	S
VLDL (mmol/L)	0.4086 ±0.232[a]	0.5065±0.089[a]	0.610 ± 0.254[b]	0.0059	S

Keys: HDL= High-density lipoprotein, TCHOL = Total cholesterol, LDL= Low-density lipoprotein, VLDL= Very Low-density lipoprotein. S=Significant at *P*=.05. PostHoc: Values within the same row with different superscripts differ significantly at *P*=.05

4. DISCUSSION

The present study investigated the effect of vitamin C supplementation on the lipid parameters of subjects exposed to petroleum for over a year. The significantly higher values observed in the lipid parameters, that is, total cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, and verylow-density lipoproteins in the fuel pump workerscompared to non-fuel pump workerssimilar to the reports of Uboh et al., [15], who reported an increase in all lipid parameters of rats exposed to petrol &kerosene fumes. Also, our findings are in line with the documentation of Ugbalaand colleagues [16]. They reported a dose dependent increase in total cholesterol and low-density lipoprotein cholesterol (LDL-C). However, their study reported a significantly lower value of triglyceride concentration which is contrary to our findings.This disparity in our results and that of Ugbala and colleagues [16] could be due to the fasting state in our study (18 hours) prior to blood collection. Ogbevire et al. [17] and Abarare et al. [18], in separate studies, reported significantly higher plasma levels of cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol in rats when exposed to premium motor spirit (PMS) fumes. In a similar study, Kapil et al. [19] and Egbuonu et al. [20] observed dyslipidaemia among workers at petroleum depots in Calabar, Nigeria,and South Haryana, India respectively occupationally exposed to PMS products and fumes. The significantly higher values of these lipid parameters indicated that exposure to gasoline vapourscould be associated with hyperlipidaemia,and therefore a risk factor for atherogenicity. The increase in LDL-cholesterol and HDL-cholesterol could be a result of gasoline-inducing cellular injury and functional abnormalities in hepatocytes by the process of lipid peroxidation since the liver plays a central role in the maintenance of lipid homeostasis.

However, when Vitamin C supplementation was introduced in an already exposed subject at a dose of 100mg daily for over a period of 60 days, the levels of cholesterol, triglycerides, HDL-C, LDL-C. and VDLD-C were significantly lowered similar to those of the control (non-attendants) and therefore no significant difference between these groups. However, those exposed without the use of vitamin C still had significantly higher values of these lipid parameters. The results observed suggest that vitamin C supplementation enhanced the anti-oxidative capacity of the system, therefore involved in the counteracting of free radicals generated via the inhalation of chemicals from petroleum fumes. This finding concurs with the observation of Gaur & Dixit, [21], who reported that the administration of C caused a significant reduction in serum total cholesterol and LDL cholesterol. More so, George-Opuda et al. [13], documented in their work that vitamin C was demonstrated as an effective anti-oxidant, particularly in oxidation-induced circumstances. Our results by implication further suggest that vitamin C at a daily dose of 100mg plays a potential role in mitigating atherosclerotic and cardiovascular risk associated with fuel toxicity through inhalation. This finding is particularly relevant for individuals exposed to occupational hazards, such as fuel pump workers, who may be at an increased risk of cardiovascular diseases due to occupational exposures. In other words, this study has demonstrated that vitamin C has a protective effect at 100mg daily against ? induced dyslipidaemia through the inhalation of petroleum fumes among workers exposed occupationally. More so, the result provides additional support for considering vitamin C as a preventive or ameliorative drug for workers exposed to petroleum fumes chronically since vitamin C is generally safe and its use is not associated with side effects when administered within recommended doses. This safety profile of vitamin C makes it a favorable candidate for preventive or ameliorative interventions, especially in occupational settings where exposures to petroleum fumes areunavoidable.

5. CONCLUSION

The study has shown that occupational exposure to petroleum fumes is associated with dyslipidaemia. However, the use of 100mg of vitamin C daily for ~~30~~ 60 days ameliorated the degree of dyslipidaemia associated with occupational exposure to petroleum fumes. Vitamin C could be considered as a preventive means to mitigate or ameliorate the adverse effects of occupational exposureto petroleum fumes and products among workers.

References

1. Elekima I, Nwachuku, EO,Ben-Chioma AE. Effect of tartrazine orally administered on the lipid profile of albino rats. European Journal of Pharmaceutical and Medical Research. 2017;4(7): 164-167
2. Elekima I. Effect of carmoisine orally administered on lipid parameters of albino rats. International Journal of Science and Research (IJSR). 2016;5(9): 861 -864
3. Elekima I,Onwuli D, Obisike UA, Aleru CP, Christian SG, Osiagor J. Effect of hibiscus sabdariffa extract orally administered on lipid parameters of albino rats. Journal of Medical Science and Clinical Research. 2016; 4 (2): 9529-9533|
4. Moro AM, Brucker N, Chara, MF, Baierle M, Sauer E,Goethel G. Biomonitoring of gasoline station attendants exposed to benzene: effect of gender. Mutation Research,2017; 813: 1-9.
5. OSHA.Chemical Sampling Information Benzene. 2011) Retrieved from Osha.gov. Retrieved on 2011-11-23.
6. Dougherty D, GarteS, Barchowsky A, Zmuda J, TaioliE. (2008).NQO1, MPO, CYP2E1, GSTT1 and GSTM1 polymorphisms and biological effects of benzene exposure —a literature review. Toxicology Letters.2008; 182(13), 7-17.
7. Carrieri M, Spatari G, Tranfo G, Sapienza D, Scapellato ML, Bartolucci GB.Biological monitoring of low level exposure to benzene in an oil refinery: Effect of modulating factors. Toxicology Letters, 2018; 298, 70-75.
8. Keenan JJ, Gaffney SH, Galbraith DA, Beatty P, Paustenbach DJ. Gasoline: a complex chemical mixture, or a dangerous vehicle for benzene exposure? *Journal of Chemical and Biological Interaction,*2010; 184(12): 293-295.
9. Badham HJ, LeBrun DP, Rutter A, Winn LM. Transplacental benzene exposure increases tumor incidence in mouse offspring: possible role of fetal benzene metabolism. Carcinogenesis. 2010; 31(6):1142-8.
10. Christian SG, Elekima I, Obisike UA, Aleru CP. Effect of petroleum on haematological parameters and lead level in fuel attendants in Port Harcourt, Nigeria. International Journal of Science and Research (IJSR). 2016; 5(3): 280 -283
11. Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, Knight AR, Taylor EL, OettrichJ, Ruskovska T, Gasparovic AC, Cuadrado A, Weber D, Poulsen HE, Grune T, Schmidt HH, Ghezzi P. Clinical Relevance of Biomarkers of Oxidative Stress. Antioxid Redox Signal.2015; 23(14): 1144-11470.
12. Ben-Chioma AE, Elekima I. Evaluation of Vitamin E and selenium levels in breast cancer patients in Port Harcourt Metropolis, Nigeria. Journal of Advances in Medicine and Medical Research. 2018; 28(2): 1-7.
13. George-Opuda MI, Adegoke OA, Bamigbowu EO. Effect of Vitamins C on Some Hematological Parameters in Albino Rats Treated with Gasoline. *Sciknow Publications*. 2014; 2(3): 55-58.

14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge.Clinical Chemistry, 1972; 18(6): 499–502
15. Uboh FE, Akpanabiatu EU, Eyoung EU, Ebong PE, Eka OU. Evaluation of Toxicological Implications of Inhalation Exposure to kerosene fumes and Petrol Fumes in Rats. Acta Biology.2005; 49, 19-22.
16. Ugbala JE, Adegoke OA, George-Opuda IM, Bamigbowu EO. Evaluation of lipid profile in male albino rats exposed to petrol fumes. International Journal of Biomedical and Advance Research. 2020; 11(2): 5321-5377.
17. Ogbevire L, Patrie O, Nathaniel M, John OD, Favour O, Ehis O,Richard O. Total Cholesterol, Triglyceride, High Density Lipoprotein Cholesterol and Low DensityCholesterol in Rat Exposed to Premium Motor Fumes. North American Journal of Medical Science, 2011; 3(6), 277-280.
18. Aberare OL, Okuonghae P, Mukoro N, Dirisu JO, Osazuwa F, Odigie E, OmoregieR. Triglycerides, total cholesterol, high density lipoprotein cholesterol and low-density lipoprotein cholesterol in rats exposed to premium motor spirit fumes. North American Journal of MedicalSciences. 2011; 3(6), 277–280.
19. Kapil S, Rakesh KS, Arun G, VedpalY. Impact of petroleum fumes on liver and kidney functioning of petrol filling attendants and garage attendants working in South Haryana, India. *European* Journal of Pharmaceutical and Medical Research.2016; 3(8):569-573.
20. Egbuonu AC, Nkwazema DC, Ezeanyika L. (2015). Cardiovascular risks and impaired lipid metabolism in asymptomatic petroleum depot workers in Calabar metropolis. Research Journal of Environmental Sciences. 2015; 9 (6), 270-279
21. Gaur GS, Dixit AK. Comparative Study of Vitamin C on Serum Lipid Profile in Healthy Male and Female Human Subjects. Journal of Scientific Research.2012; *4*(3), 775–781.
~~22.~~

Formatted: Indent: Left: 0.55", No bullets or numbering