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Changes in Antioxidant and Energy Metabolism Parameters in Men in Ebocha, Niger Delta, Nigeria from Chronic Exposure to Gas Flaring

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Abstract: The impact of pollution due to gas flaring on men native to Ebocha in the Niger Delta Area was investigated. Blood specimens from one hundred (100) apparently healthy men from Ebocha community aged 30 to 50 years were screened. Control blood specimens were obtained from one hundred (100) apparently healthy men from Uturu community [a location with no history of petroleum hydrocarbon (PHC) pollution]. Standard analytical procedures were used to determine the concentrations of antioxidants in blood specimens such as glutathione (GSH), ascorbic acid (AA) and malondialdehyde (MDA). The activities of energy metabolic enzymes such as lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) were also assayed. Results obtained revealed that mean values of concentrations of GSH (3.94±0.18 µmol/l) and AA (94.8±18 µmol/) for men from Ebocha were significantly ($p<0.05$) lower (4.99±18 mg/dl) and (145.7±23 mg/l) than those of men from Uturu respectively. In contrast, the mean value of MDA concentration for men from Ebocha (4.04±0.38µmol/L) was significantly ($p<0.05$) higher than that of men (3.30±0.65 µmol/L) from Uturu. The mean activities of LDH (388.5±64 IU/L) for men from Ebocha were significantly ($p<0.05$) higher than those of men (229.8±25 IU/L) from Uturu. The mean activities of G-6-PDH for men from both Ebocha and Uturu communities were essentially similar. These findings show that GSH, AA, MDA concentrations and LDH activity in the blood specimens of men from Ebocha were altered and it is possibly due to the chronic exposure to gas flaring in Ebocha community.

Keywords: Chronic exposure, Energy metabolism, Antioxidant, Gas flaring, Ebocha, Uturu, Niger Delta

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Introduction

Oil and gas exploration and exploitation started in the Niger Delta Region of Nigeria in 1956, when the first commercial oil well was discovered at Oloibiri in the present day Bayelsa State in the Niger Delta area of Nigeria (World Bank, 1995). A delta is a region where a river enters into an ocean body with numerous distributaries. Such an ocean is usually low lying periodical inundation. Its vegetation is luxuriant varying from fresh water to brackish mangrove. The soil is predominantly clayey with low content of organics which invariably makes it highly fertile (Onwuama, 1997). Due to its swampy nature, high acidic and sordid condition, it constitutes a regional environmental challenge. The Niger Delta region encompasses over 20,000 km^2 in Southern Nigeria. It is the centre of oil and gas production and allied activities in Nigeria (World Bank, 2012). It is the richest part of Nigeria in terms of natural resources such as oil and gas deposits, extensive forests, suitable agricultural lands and abundant aquatic resources (Argo, 2002). Oil is associated with the economic development of Nigeria. Between 1976 and 1977, crude oil provided roughly 92% of Nigeria's total export earnings. Crude oil has continued to be the major income earner for the country since the shift of attention from the agricultural sector (Imevbore and Adeyemi, 1981). The importance and impact of oil to Nigeria can be fully appreciated if one realizes the dominant role it plays in the economy by providing job opportunities and economic development. However, the region's potential for sustainable development remains unfulfilled and its future is being threatened by diverse environmental pollution problems (Imevbore and Adeyemi, 1981).

An on-site visit to Ebocha in Niger Delta revealed the deteriorating condition of the environment. The free disposal of gas through flaring generates tremendous heat that causes increased water loss by transpiration in nearby plants resulting in wilting and death. Flared gas is the most significant source of air pollution from oil and gas installation (Kindzierski, 2000). During most of these activities in the oil and gas industries, waste either in solid or gaseous form are generated and discharged into the environment (Gobo *et al.*, 2009). Flared gas is one of the generated wastes in the oil and gas industry. Gas flaring is a common practice of burning off of flammable gases through combustion non-premixed flame in an open atmosphere (Ikoro, 2003). Gas flaring is one anthropogenic activity which results in emission of green house gases that cause global warming, disequilibrium of the earth, unpredictable climate changes and major natural disaster. Gas flaring has potential harmful effects to humans and plants, and deteriorates the physical environment as it releases a variety of poisonous chemicals including nitrogen dioxide, sulphur dioxide, carbon monoxide, methane, particulates, and volatile organic compounds like benzene, xylene, hydrogen sulphide as well as carcinogen like dioxin (Ajugwo, 2013). Humans exposed to such substances can suffer from respiratory problems such as asthma, breathing difficulties, pain and chronic bronchitis. Occupational exposures to petroleum fumes have been reported to have toxic effects on various organs and systems, and these include immune and nervous systems (Smith *et al.*, 1993; Ovuakporaya *et al.*, 2012). Also, organs such as the heart, liver, lungs, skin and kidneys are affected by these toxic substances resulting in various diseases and different forms of genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestations (Ovuakporaya *et al.*, 2012).

Furthermore, discussions with elderly people on their experience in the Ebocha area revealed that there is disappearance of endangered species in plants such as guava, plantain and banana; animals such as frog, millipede, earthworm and grasshopper. One effect of the intense heat from the gas flare stations is reduced crop yield (Atuma and Ojeh, 2013). They complained that because they live and work alongside the flares inhale toxic gas flare pollutants with no protection which result to diverse health abnormalities such as asthma, headaches, drowsiness and high body temperature have often times affected them (Okeke *et al.*, 2016). The aim of the present study was to investigate the effect of chronic exposure to gas flaring on the antioxidant and energy metabolism parameters of men native to Ebocha, in the Niger Delta area of Nigeria.

Materials and Methods

Study area:

The study was conducted at two different locations in the Niger Delta area of Nigeria (Fig. 1). Ebocha, an oil and gas-producing area with active

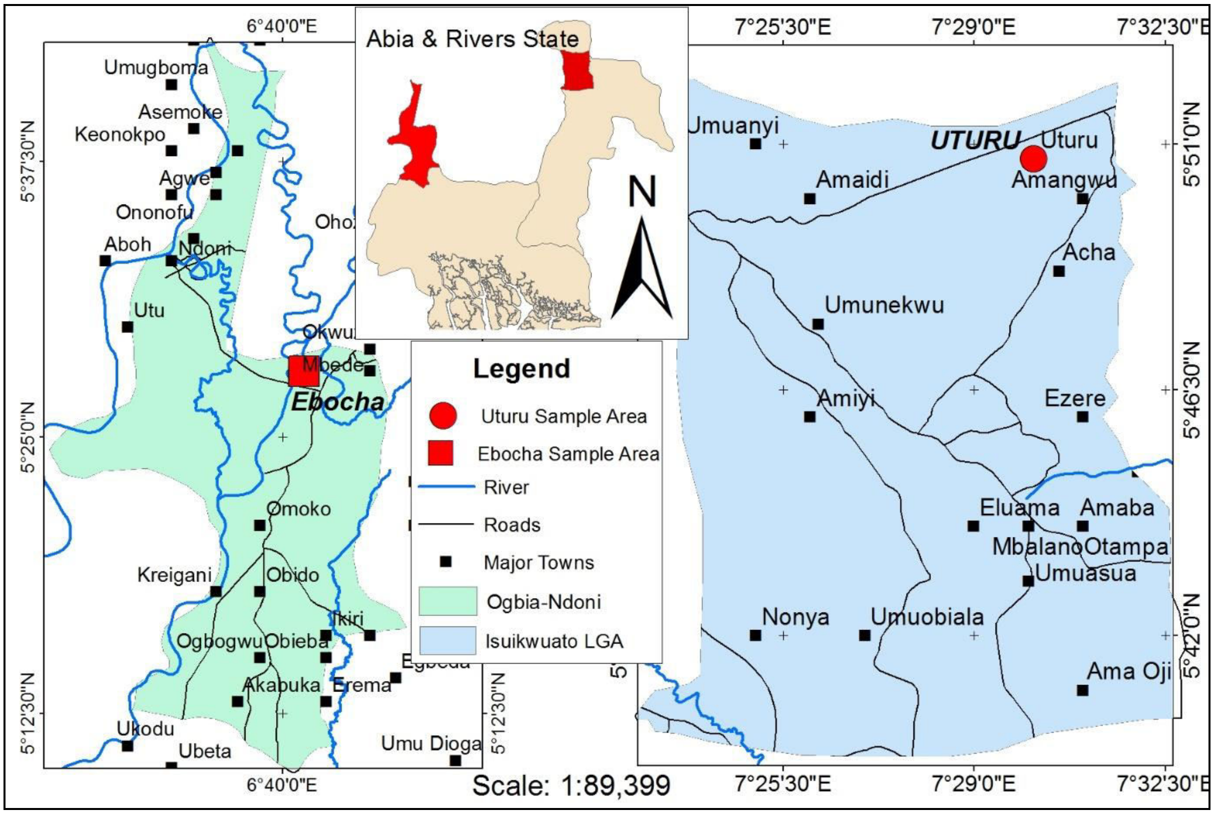

Fig. 1: Map of Ebocha and Uturu in Niger Delta, Nigeria.

gas flaring operation by one of the major oil exploration and production company in Nigeria served as test location. Oil exploration and production have been on in the area for over forty years (NNPC, 1997). The test location has twenty five families living 5 km within the flaring site while twenty five families from Uturu, a location with no history of petroleum hydrocarbon (PHC) pollution served as control. The residents of both communities are mainly farmers, and share many common characteristics such as age and food. The study was carried out during the dry season, between December and March, 2015. The season was chosen as we are studying chronic effect and more so studies had been carried out on the air quality of Ebocha environment during both dry and rainy seasons (Nwaogu and Onyeze, 2010).

Selection of subjects for the study:

One hundred (100) apparently healthy male farmers aged 30 to 50 years who have lived in Ebocha continuously for at least twenty (20) years within the immediate environment (5 km) from the gas flaring location were eligible as subjects for the chronic toxicity study. One hundred (100) aged-matched male farmers who have consistently resided in Uturu for over twenty (20) years were enlisted as control. Eligible subjects for the study were selected based on the administration of consent form and questionnaires and those who were within our inclusion criteria, which include those who were apparently healthy, without any known sickness such as HIV, tuberculosis, malaria, hepatises B, diabetics and have consistently lived in the study areas for twenty years. The essence and procedures of the study were thoroughly explained to the candidates and only those who consented to in writing and /or thumb printed in our questionnaire participated in the study.

Ethical Approval:

The research was approved by the Ethical Committee on Human Research of the Department of Biochemistry, School of Biological Sciences, Federal University of Technology, Owerri, Nigeria with Approval No. FUT/SOBS/BCH/COM.1/ 012/2017 and the study conforms to the Helsinki Declaration on Medical Research.

Collection of blood specimen:

Six millilitres (6 ml) of venous blood specimens were collected from each subject, (4 ml) was dispensed into ethylene diamine tetra acetic acid (EDTA)-containing specimen bottles while (2 ml) was dispensed into plain specimen bottles and taken to the Research Laboratory, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. Four millilitres (4 ml) blood specimen was allowed to clot, the serum collected, and analysed same day for the antioxidant parameters and energy metabolic enzymes - lactate dehydrogenase (LDH) and Glucose-6-phosphate dehydrogenase (G-6-PDH), respectively. Two millilitres (2 ml) of the blood plasma specimen was used to assay Glucose-6-phosphate dehydrogenase. This was done for both blood sample specimens from Ebocha in the Niger Delta Area in Rivers State State and Uturu, in Abia State.

Determination of concentrations of antioxidants:

Glutathione concentration was determined using the method of Pastore *et al.* (2003). The concentration of ascorbic acid was determined using the method according to Roe and Kuether (1961). Malondialdehyde concentration was determined using the method of Varshney and Kale (1990).

Assay of the activities of energy metabolic enzymes:

Lactate dehydrogenase activity was assayed using colorimetric method following the method of Wilkinson (1976). Glucose-6-phosphate dehydrogenase activity was also assayed using the modified method of Wilkinson (1976).

Statistical analysis:

Data generated were analysed using a one way Analysis of Variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) version 20 running on Windows PC. Data for each parameter were expressed as mean ± Standard deviation. The significant difference between the test means and control means were determined at $p<0.05$.

Results

The mean concentration of glutathione was significantly ($p<0.05$) lower for the men from Ebocha than for the men from Uturu (Fig. 2), though the mean values obtained are within normal range. The reason for the lower value obtained for the men from Ebocha is that the antioxidant glutathione was employed to mop up free radicals in men from Ebocha due to pollution in that environment due to gas flaring.

The mean value for ascorbic acid concentration (Fig. 3) of men from Ebocha were found to be significantly ($p<0.0.5$) lower than that obtained for men from the non-polluted area of Uturu. This reduction in ascorbic acid concentration in men from Ebocha, is attributable to the higher pollution level in that environment.

The results obtained for malondialdehyde concentration (Fig. 4) revealed that the mean concentration of malondialdehyde was significantly ($p<0.05$) higher for the men from Ebocha than for the men from Uturu. Although the obtained values were within normal range, the higher mean value obtained for individuals from Ebocha may be an adaptive response to pollution due to gas flaring. It also indicated that there was more of lipid peroxidation probably as a result of free radical bombardment due to gas flaring in men from Ebocha.

The mean lactate dehydrogenase activity (Fig. 5) was significantly ($p<0.05$) higher in the men from Ebocha than for those in men from Uturu.

The results obtained for glucose-6-phosphate dehydrogenase activity (Fig. 6) revealed slightly higher in men from Ebocha though there was no significant difference ($p<0.05$) in the mean activity for the men from Ebocha which was similar to that obtained for the men from Uturu. The similar mean activities obtained for individuals from Ebocha and Uturu revealed that there was no

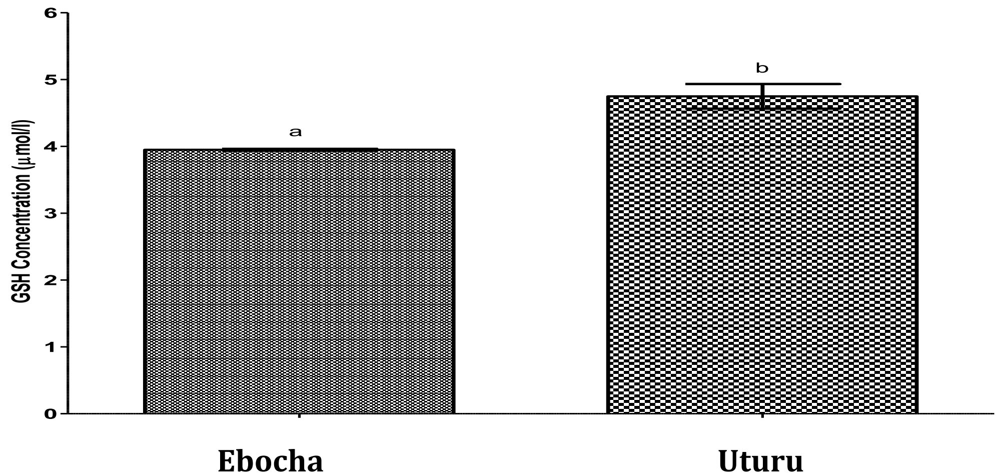

Fig. 2: Mean values of glutathione concentrations. Bars represent mean ± SD of three determinations. Bars with different superscript letters are statistically significant (p<0.05).

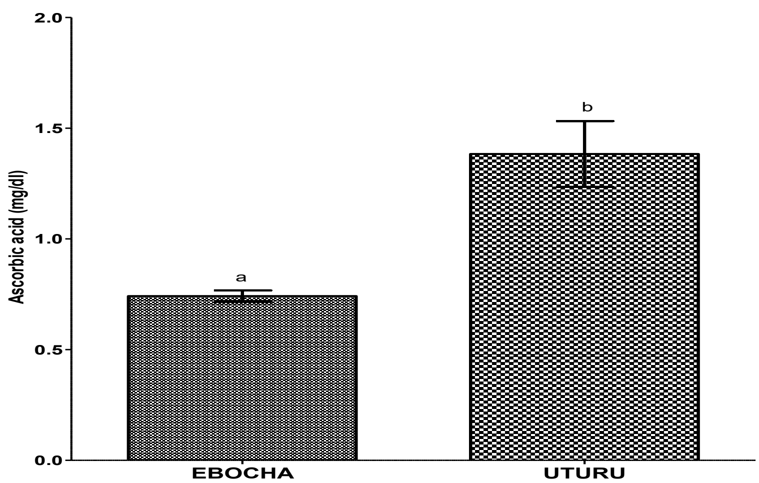

Fig. 3: Mean values of ascorbic acid concentrations. Bars represent mean ± SD of three determinations. Bars with different superscript letters are statistically significant (p < 0.05).

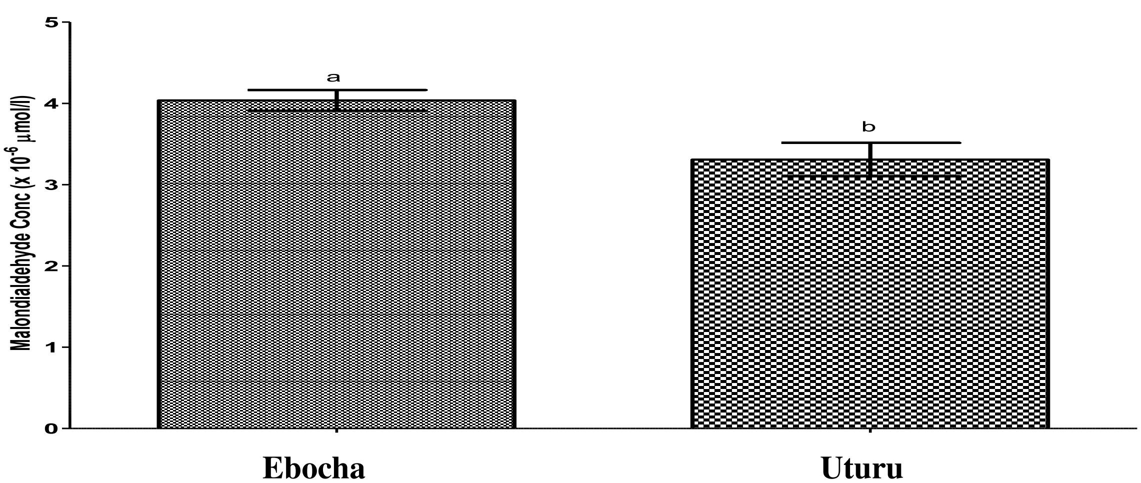

Fig. 4: Mean values of malondialdehyde concentrations. Bars represent mean ± SD of three determinations. Bars with different superscript letters are statistically significant (p < 0.05).

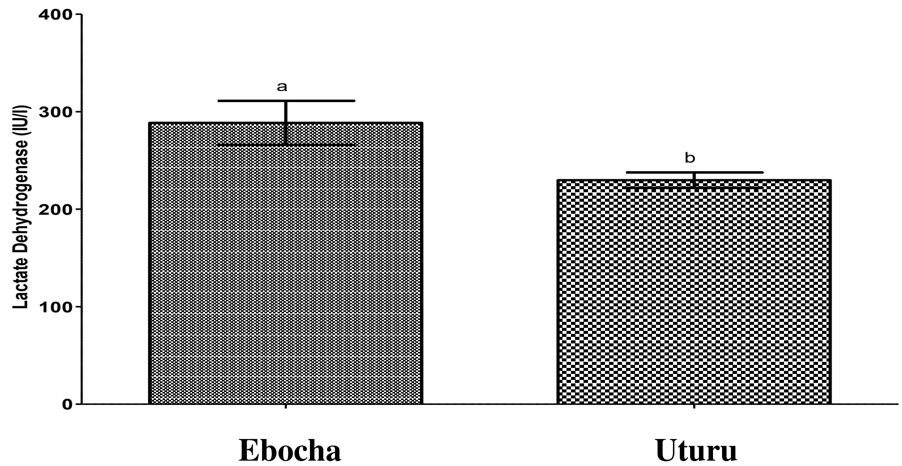

Fig. 5: Mean values of lactate dehydrogenase (LDH) activity. Bars represent mean ± SD of three determinations. Bars with different superscript letters are statistically significant (p<0.05).

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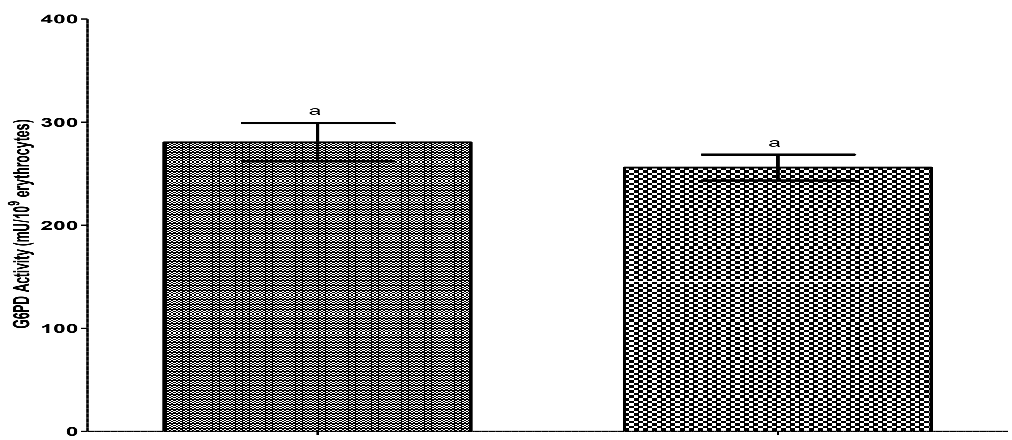

Fig. 6: Mean values of glucose-6-phosphate dehydrogenase (G-6PDH) activity. Bars represent mean ± SD of three determinations. Bars with different superscript letters are statistically significant (p<0.05).

enzyme deficiency, infection or drug-induced haemolysis. However, the slight higher mean activity obtained for the men from Ebocha may indicate that there was continual utilization of reducing potentials (NADPH) probably by haemoxygenase for bilirubin synthesis and for reduction of oxidized glutathione to glutathione, and for mopping up of free radicals.

Discussion

When an individual is exposed to high concentrations of xenobiotic, more concentrations of glutathione is utilized for conjugation–a key step in the body's detoxification process thereby making it less available to serve as an antioxidant. The mean concentration of glutathione for the men from Ebocha was significantly (p<0.05) lower than that of men from Uturu. This result is in accordance with that of Niki *et al*. (1982), who reported that glutathione helps to regenerate some important antioxidants especially vitamin E used to scavenge free radical. Glutathione is sensitive to environmental pollutants, hence used as marker for the toxic effects of ingestion or inhalation of various xenobiotics. The observed difference in glutathione in the individuals from Ebocha and Uturu might be due to the antioxidant function of glutathione by which it scavenges free radicals due to gas flaring in Ebocha.

Ascorbic acid is a water soluble potent antioxidant molecule which scavenges free radicals in the soluble cytoplasm and aqueous intercellular environment (McKee and McKee 1999). As a powerful cellular reductant, it readily donates electrons to reduce free radicals in the cellular compartment and is converted to dehydroascorbic acid (DHAA), its oxidized form. Gas flaring in Ebocha may have resulted in the significant ($p<0.05$) reduction of the mean concentration of ascorbic acid in men living in that environment when compared to the value obtained for men from Uturu. This finding is similar to the observation of Niki *et al.* (1982), who reported a substantial reduction in liver ascorbic acid concentration and function in print workers exposed to toluene which is also a constituent of gas flare. This observed marked reduction in ascorbic acid concentration is probably due to pollution in the environment which could be attributed to the involvement of ascorbic acid in reactions with reactive intermediates, thereby reducing its concentration.

Mean concentration of malondialdehyde (MDA) for the men from Ebocha was significantly ($p<0.05$) higher than those in men from Uturu. Also, cadmium, a major heavy metal constituent of gas flare and from fuel combustion, can be inhaled or consumed from food crops such as rice, potato and leaves of vegetables, fruits and oily seeds (Ogidiolu, 2003; Idiodo-Umeh and Ogbeibu, 2010). It leads to generation of reactive oxygen species (ROS) that causes an increase in lipid peroxidation. Hence, this observation is similar to that of Egwuregwu *et al.* (2013, who reported an increase in MDA concentration in blood indices in humans exposed to gas flares over a long period.

Lactate dehydrogenase catalyses the conversion of pyruvate to lactate with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide (NAD^+). The mean activity of lactate dehydrogenase of the men from Ebocha was significantly ($p<0.05$) higher than that in men from Uturu. The effort to adapt to the pollution may be responsible for this observation. This observed result is similar to that of Apple and Rogers (1986), who reported an increase in skeletal muscle lactate dehydrogenase activity in men and women marathon runners. Lactate dehydrogenase catalyses the conversion of pyruvate to lactate under stressed conditions. The result of the assay of lactate dehydrogenase activity reflects the metabolic capacity of tissues, after long term exposure to polluted environment. The liver among other tissues is one of the richest sources of lactate dehydrogenase and leakage of the enzyme from affected liver tissue can increase the observed activity to a significant extent. This observed result might be attributed to the direct toxic effects of gas flare pollutants on hepatocytes, since the liver is the principal organ responsible for detoxification in vertebrates. In addition, this might be as a result of a shift from aerobic to anaerobic metabolism due to hypoxia, under free radical-producing stress conditions in Ebocha, a petroleum hydrocarbon-polluted environment.

There was a slight increase in glucose-6-phosphate dehydrogenase activity for men from Ebocha. However, the values obtained showed that there was no significant ($p< 0.05$) difference between the means of glucose- 6-phosphate dehydrogenase activity in men from Ebocha and Uturu. The observed result might be as a result of defence mechanism against oxidative damage in red blood cells which is particularly dependent on glucose–6–phosphate dehydrogenase activity. According to studies, oxidative stress may alter glucose–6–phosphate dehydrogenase activity (Mason, 1996). Also, the values obtained might be due to continual utilization of reducing potential (NADPH) by haemoxygenase (HMOX) as a reducing agent for bilirubin synthesis and for reduction of oxidized glutathione to glutathione used for mopping up free radicals in a living system (Olufunsho *et al*., 2014; Miki *et al*., 1982). Furthermore, the slight increased activity in glucose-6-phosphate dehydrogenase obtained in this study might reflect metabolic imbalance due to pollution stress in direct relation to the limited availability of reducing potential (NADPH).

Conclusion

The present study revealed that chronic exposure to gas flaring in Ebocha in the Niger Delta significantly ($p<0.05$) reduced the concentrations of glutathione and ascorbic acid but markedly increased the concentrations of malondialdehyde. There was also a significant ($p<0.05$) increase in LDH activity while the activity of G-6-PDH of men were essentially the same in both the polluted studied area and the control location. The study also revealed that chronic exposure to gas flaring in Ebocha altered the antioxidant indices investigated as well as the energy metabolic enzyme LDH in men residing in that area who are predominantly farmers. This farming activity exposes them to gas flare. The study calls for immediate governmental policies to reduce gas flaring activity in the area and to make the area habitable for the residents.

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