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Toxicological assessment of crude oil vapour and the modulating effect of vitamin E on the heart of albino rats

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Abstract

This study investigates the effect of vapour of various concentrations of crude oil and oral co-administration of vitamin E on the heart of albino rats. Thirty (30) albino rats were randomly grouped into six (6) of five (5) rats each and designated A to E. Rats neither exposed to crude oil vapour nor oral administration of vitamin E, rats exposed to oral administration of 15 mg/kg bw vitamin E but not exposed to vapour of crude oil were designated groups A and B. Rats exposed to oral co-administration of vitamin E and vapour of 25%, 50%, 75% and 100% crude oil were designated groups C, D, E and F respectively. Rats' treatment was over a period of 28 days, after which the rats were euthanized for biochemical analyses. Serum total protein (TP), total cholesterol (CHOL) and triglycerides (TRIG) levels were determined. Standard enzyme assay was conducted for alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase and lactate dehydrogenase (LDH) of the heart. Generally, the concentration of TP of serum of other groups of rats, except group B, was significantly higher relative to the Control ($p \leq 0.05$). Serum CHOL and TRIG levels increased as the concentration of crude oil increased. Heart AST activities of rats in groups A, B and C were not significantly different ($p \leq 0.05$), similarly the AST activities of heart of rats in groups D, E and F were not significantly different ($p > 0.05$). There was no significant difference between the gamma glutamyl transferase (GGT) activity of Control rats not exposed to crude oil vapour and not given oral administration of vitamin E (A) and the Control in Group B (given oral administration of vitamin E but not exposed to crude oil vapour). The specific activity of lactate dehydrogenase (LDH) of heart of both control rats and those of rats exposed to the vapour of various concentrations of crude oil were not significantly different ($p > 0.05$). Experimental evidence from this study revealed that vapour of crude oil can induce biochemical changes in the heart of rats while oral co-administration of vitamin E could reduce the risk posed by exposure to vapour of crude oil by 50%.

Keyword: Toxicological assessment; Crude oil; Vapour; Vitamin E; heart

1. Introduction

Crude oil is popularly known all over the world and its products are in high demand. Apart from being of high demand in industries all over the world (Herrera et al., 2018), it has been globally recognised as one of the major sources of pollution in terrestrial (Rajabi and Sharifipour, 2019), atmospheric (Afshar-Mohajer et al., 2020) and in the ocean (Afshar-Mohajer et al., 2020). According to OPEC (2019) the crude oil reserve in global inventory was estimated to be greater than 1000 billion barrels (bb) in 1997 and 1498 bb at the end of 2018 (almost 44% during the last 20 years). This is an indication that the world is in need of more crude oil for material, energy, manufacturing and transportation activities. Crude oil exploitations as an upstream operations is comprised of various steps of exploration, extraction, storage, transportation and refinement (Masnadi et al., 2018b) and these are capable of negatively affecting human health and the environment through spillage emissions that are hazardous (Ramirez et al., 2017). Some of these hazardour hydrocarbons can be very severe. The more the oil processing activities, the more is the severity of hydrocarbon contamination released to the environment (Zhang. et al., 2020). Oil spillage is one of the main sources of

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environmental pollution in the business of exploration. Malfunctioning of equipment and sometimes lackadaisical attitude of human categorized as human error and unavoidable hazardous emissions when oil mass is open-to-air without any control measures are the ways through which spillage occurs (Pelta et al., 2019). Of all the error sources, human error has been noted to be the main reason of all the accidental oil spills worldwide. In 2018, about 70% was said to have happened in shipboard operations. This indicates that the more crude oil is being shipped, the more is the number of spillage (ITOPF, 2018). The consequence of this spillage on the operating environment is enormous. Moreover, the negative effects of crude oil pollution goes beyond the operating environment because , some of the hydrocarbons emitted are volatile because they have very low boiling point which makes them escape to easily diffuse into the atmosphere and spread to remote environments, thereby creating environmental health issues.

VOCs are emitted in so many ways and stages of oil productions. During the various steps of the crude oil industry's activities, starting from the extraction sites through transportation facilities and to storage tanks and refineries, volatile compounds can escape from the oil mass. For the above reasons, apart from the transportation sector giving rise to vehicle exhaust pollution, the production sites and oil refineries have been recognised to be the second major origins of the VOCs (Khoramfar et al., 2018)The composition of crude oil is such that about 85% of the crude oil compounds consists of hydrocarbons with an average of 30% of alkanes, 49% cycloalkanes, 15% aromatics, and 6% asphaltics (Light HCs in crude oil) which can easily escape into the air due to their high vapour pressures and low boiling points to form the main part of the atmospheric VOCs. Moreover, on a global scale, VOCs can produce photochemical ozone (O_3) and other harmful oxidants which adversely affect the air quality and human health (Mustafa et al., 2018). In addition to methane, some other alkanes (ethane, propane, butane, pentane, and hexane) emitted from crude oil, are capable of interacting with NOx in the air forming tropospheric ozone. ''The Intergovernmental Panel on Climate Change (IPCC) has found that emissions from fossil fuels are the dominant cause of global warming. In 2018, 89% of global carbon dioxide emissions came from fossil fuels and industry''(ClientEarth, 2022). So, crude oil emissions are also considered as ozone precursors and global warming agents.

Nigeria has been a major oil producing country and environmental pollution due to gas flaring and spillage from petroleum products have been reported on several occasions (Anejionu, et al., 2015; Akakuru et al., 2017; Checkley et al.,, 2021). Petroleum products contain hydrocarbons(HCs) and hydrocarbons are toxic (Gramigni et al., 2021). Over the last three decades, cardiovascular diseases and mortality have been on the rise (Adedapo, 2017). Cardiovascular diseases are observed from all ages of human from infants to adults. Meanwhile, humans use the petroleum in many forms and some are directly involved in the business of petroleum dispensing such as at the filling stations. The fume constantly being inhaled from petroleum is likely to affect these workers. It therefore becomes imperative to find out the consequences of such inhalation.

The purpose of the study is to find out the toxic level to rats' cellular system and modulating effect of vitamin E with a view to making inference about the possibility of such effect on humans.

2. Material and method

2.1. Reagents

Reagents and solvents were of analytical grade and are products of British Drug House, Poole, England.

2.2. Experimental rats and treatments

Thirty adult Albino rats (*Rattus novergicus*) were obtained from the Animal Holding of the Department of Anatomy University of Benin, Benin-City, Nigeria. The experimental animals were kept inside 6 plastic cages containing 5 animals each. The rats were classified into 6 groups as shown in Table 1.

2.3. Anaesthetisation of Animals and Isolation of Tissues

The rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture. The tissues (liver, kidney, stomach) of the animals were removed into a beaker containing ice cold 0.25 M sucrose solution. The blood was obtained through their jugular veins. Each blood sample was thereafter centrifuged at 3,500rpm for about 15 minutes using refrigerated centrifuge RC650s and the serum obtained was preserved at -8 ˚C until required for use.

2.4. Preparation of Homogenate

The isolated tissues were weighed and a portion of each tissue was cut out, chopped into very small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenates were diluted using 0.25 M sucrose solution to the tune of 1 in 30 dilutions. The diluted homogenates were stored at temperature of -8^{o}C until required for use.

Table 1 Experimental rats and treatments over a period of 30 days

Groups	Treatments
Group A	Control, no exposure to vapour of crude oil and no oral administration of vitamin E
Group B	No exposure to vapour of crude oil but treated + oral administration of 15 mg/kg bw vitamin E
Group C	Exposure to vapour of 25% v/v crude oil + oral administration of 15 mg/kg bw vitamin E
Group D	Exposure to vapour of 50% v/v crude oil + oral administration of 15 mg/kg bw vitamin E
Group E	Exposure to vapour of 75% v/v crude oil + oral administration of 15 mg/kg bw vitamin E
Group F	Exposure to vapour of 100% v/v crude oil + oral administration of 15 mg/kg bw vitamin E

2.5. Enzyme assay and Serum Biochemistry

The cholesterol concentration in the serum of the rats placed on leachate and leachate-contaminated groundwater samples was determined following the method described by Abell et al (1958). Cholesterol reacts with acetic acid/acetic anhydride mixture in the presence of concentrated sulphuric acid to give a green colour. The absorbance of the colour at 570nm which is proportional to the amount of cholesterol in the sample is read on a spectrophotometer. Serum triglycerides concentration was determined following the method described by National cholesterol Education Programme (NCEP) (James, 2001). The protein concentration in the tissue of experimental rats was determined following the method reported by Gornal et al (1949). Cupric ions in alkaline solution form a purple coloured complex with any compound containing repeated-CONH-links such as proteins. The purple colouration is due to the coordination between the cupric ions and the unshared electron pair of peptide nitrogen and the oxygen of water to form the colour coordination complex. The activity of AST and ALT in the serum and tissues of experimental animals was determined following the procedure reported by Reitman and Frankel (1957) as modified by Schmidt and Schmidt (1963). The activity of Gamma glutamyl transpeptidase (GGT) was determined following the method described by Tietz (1990). In this method GGT transfers glycylglycine to L-γ-glutamyl-p-nitroanilide to form L-γ-glutamylglycylglycine and p-nitroaniline. The method used for assaying lactate dehydrogenase is based on that of Wroblewski and La Due (1955) in which pyruvate is reversibly reduced to lactate in the presence of nicotinamide adenine dinucleotide (reduced) as co-enzyme. The oxidized form of co-enzyme does not absorb at 340nm while the reduced form absorbs strongly. The reaction was followed by measuring the rate of loss of extinction at 340nm.

3. Results

Table 2 presents the specific activity of selected enzymes of heart of rats exposed to vapour of various concentrations of crude oil and oral administration of Vitamin E. The specific activity of lactate dehydrogenase (LDH) of heart of both control rats and those of rats exposed to the vapour of various concentrations of crude oil were not significantly different ($p>0.05$). There was no significant difference between the gamma glutamyl transferase (GGT) activity of Control rats not exposed to crude oil vapour and not given oral administration of vitamin E (A) and the Control in Group B (given oral administration of vitamin E but not exposed to crude oil vapour). Notably, GGT activity of rats in group B was not significantly different ($p>0.05$) from rats in groups C and group D (exposed to vapour of 25% and 50% crude oil respectively and oral administration of vitamin E). Similarly, GGT activities of heart of rats exposed to vapour of 50% (D), 75% (E) and 100% (F) were not significantly different ($p>0.05$). However, the heart GGT activities of rats exposed to vapour of various concentrations of crude oil were significantly higher ($p\leq$) relative to that of Control (A). Specifically, heart GGT of rats exposed to vapour of various concentrations of crude oil was about two (2) folds that of Control (A). Heart AST activities of rats in groups A, B and C were not significantly different ($p\leq0.05$), similarly the AST activities of heart of rats in groups D, E and F were not significantly different ($p>0.05$). However, heart AST activities of rats in groups D, E and F were significantly higher ($p\leq0.05$) relative to the control (A). It is noteworthy that there was no significant difference ($p>0.05$) between the enzyme activity of heart of rats in groups A and B, except ALT activity.

Table 2 Specific activity (U/L/mg protein)) of selected enzymes of heart of rats exposed to vapour of crude oil and oral administration of Vitamin E

RAT GROUPS	LDH	GGT	ALT	AST
A	13.45±6.18^{a}	0.48±0.11^{a}	0.40±0.10^{a}	2.40±0.32^{a}
B	14.76±5.91^{a}	0.60±0.41^{ab}	0.63±0.05^{b}	2.71±0.35^{a}
C	15.58±3.11^{a}	0.71±0.20^{b}	0.80±0.21^{b}	2.80±0.12^{a}
D	15.75±4.80^{a}	0.97±0.22^{bc}	2.14±0.17^{c}	3.18±0.44^{b}
E	18.04±1.64^{a}	1.06±0.28^{c}	2.48±0.11^{c}	3.30±0.31^{b}
F	18.59±9.15^{a}	1.35±1.31^{c}	3.41±0.42^{d}	3.56±0.12^{b}

Column bearing different superscripts are significantly different (P≤0.05). Tabulated data are means of three (3) determinations ± SEM.

Concentration (mg/dL) of serum total protein (TP), total cholesterol (CHOL) and triglyceride (TRIG) of rats exposed to vapour of crude oil and oral administration of Vitamin E is shown in Table 3. Generally, the concentration of TP of serum of other groups of rats, except group B, was significantly higher relative to the Control (p≤0.05). Serum CHOL and TRIG levels increased as the concentration of crude oil increased. However, concentrations of serum CHOL of rats in groups B, C and D were not significantly different compared to the Control (p>0.05). Conversely, serum CHOL level of rats exposed to vapour of 75% and 100% crude oil were significantly higher (p≤0.05) than the Control. Serum concentration of rats exposed to vapour of 100% crude oil, specifically, is about 160% that of Control. Concentrations of serum TRIG of rats in groups A, B and C were not significantly different (p>0.05). The concentration of serum TRIG of rats in group C is worthy of mention as it was not significantly different (p>0.05) from groups A, B, D and E. Conversely, the serum TRIG of rats exposed to vapour of 100% crude oil was about two (2) folds that of Control.

Table 3 Concentration (mg/dL) of serum total protein (TP), total cholesterol (CHOL) and triglyceride (TRIG) of rats exposed to vapour of crude oil and oral administration of Vitamin E

RAT GROUPS	TP	CHOL	TRIG
A	4.81±0.19^{a}	319.70±48.66^{a}	252.92±61.47^{a}
B	5.16±0.30^{ab}	321.95±50.80^{a}	300.27±42.26^{a}
C	5.25±0.19^{b}	338.69±46.49^{a}	341.04±46.30^{ab}
D	5.31±0.52^{b}	363.05±107.36^{ab}	405.44±79.19^{b}
E	5.42±0.35^{b}	433.83±132.59^{bc}	407.98±113.62^{b}
F	5.50±0.06^{b}	570.83±63.39^{c}	743.52±130.57^{c}

Column bearing different superscripts are significantly different (P≤0.05). Tabulated data are means of three (3) determinations ± SEM.

4. Discussion

The Niger-Delta region of Nigeria is an oil rich area so much so that the major occupation of people of the area is in the oil and gas sector. The oil and gas sector is so wide that it can accommodate almost every service provider. Consequently, almost everyone in the environment of oil exploration, refining, transportation and marketing are exposed to the vapour of crude oil. Vapour of petroleum products has been reported to produce some harmful effects (Adeyemi and Isukuru, 2017; Adeyemi and Adeyemi, 2020); nevertheless the vapour is unavoidable to the people who earn a living by working in the oil and gas sector. The present study is an attempt to provide a strategy for ameliorating the effect of crude oil vapour on those who are being exposed regularly. Co-administration of vitamin E was used in this study and rats were the models employed.

Data from this study as expressed in Table 3 suggested that vapour of crude oil is capable of triggering biochemical responses in the heart of rats. These responses could be abnormal enzyme activities. LDH activity of heart was exempted from the effect of vapour of crude oil. The reason for unhindered LDH activity is not clear, but it could not be unconnected to vitamin E. Activities of other enzymes of the heart of rat studied (GGT, AST and ALT) revealed that at 25% crude oil, the effect of the crude oil vapour was suppressed by the oral administration of vitamin E. Oral

administration of vitamin E was also implicated in the modulation of effect of crude oil vapour in such a way that beyond 50% concentration, crude oil vapour exerts no significant effect relative to 50% of its concentration.

GGT is a cell surface enzyme that hydrolyzes the gamma-glutamyl bond of extracellular reduced and oxidized glutathione, initiating their cleavage into glutamate, cysteine (cystine) and glycine. GGT is normally expressed on the apical surface of ducts and glands, salvaging the amino acids from glutathione in the ductal fluids. GGT, which is localized to the cell surface, cleaved extracellular GSH, thereby providing the cell with the amino acids necessary for intracellular GSH synthesis. GGT activity enabled the cells to maintain their intracellular GSH levels, thus resisting the toxicity of cancer promoting compounds and enabling them to respond to the proliferative signals triggered by the carcinogenic regimen. Now, decades later, there is a great deal of new information about the enzyme that supports this hypothesis (Adeyemi and Adeyemi, 2023). Alanine aminotransferase (ALT) is a transaminase enzyme that was formerly known as serum glutamate pyruvate transaminase (SGPT). Alanine aminotransferase catalyzes the transfer of an amino group from alanine to alpha-ketoglutarate in the alanine cycleto form pyruvate and glutamate. The ALT enzyme is found in serum and organ tissues, especially liver, although significant concentrations are also found in kidney, skeletal muscle, and myocardium. Aspartate aminotransferase (AST) is a transaminase enzyme that catalyzes the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate. The AST enzyme was formerly known as serum glutamate oxalate transaminase (SGOT) and is present inall tissues except bone, with highest levels in liver and skeletal muscle. Concentration of AST is elevated after bruising, trauma, necrosis, infection, or neoplasia of liver or muscle. The AST enzyme is found in cerebrospinal fluid, exudates, and transudates in proportion to the amount of cellular damage.

Serum biochemicals revealed that exposure to crude oil vapour is a concern. Levels of serum total protein (TP) indicated that vitamin E probably modulates toxicological effect of crude oil vapour. Serum total cholesterol (CHOL) lends credence to the chemo-protective role of vitamin E as significant changes occurred upon exposure to vapour of crude oil at concentration above 50%. It is not apparent why serum triglycerides (TRIG) of rats exposed to vapour of 100% crude oil was about 200% that of Control.

Cholesterol is a lipid synthesized by virtually all cells, especially the liver. It functions as structural component of membranes, precursors of bile salts, steroid hormones and vitamin D. Cholesterol in animal tissues is the most abundant member of a family of polycyclic compounds known as steroids. Although, many steroids are produced by the testes and the ovaries, the two most important are testosterone and estradiol. These compounds are under tight biosynthetic control, with short and long negative feedback loops that regulate the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary and gonadotropin releasing hormone (GnRH) by the hypothalamus (Ahmad, 2020).

5. Conclusion

In conclusion, experimental evidence from this study revealed that;

Vapour of crude oil can induce biochemical changes in the heart of rats

- Effect caused by vapour of crude oil is a function of its concentration or rather oil to water ratio
- Co-administration of therapeutic dose of vitamin E was able to modulate the toxic effect caused by crude oil vapour up to 50% v/v of crude oil
- Lactate dehydrogenase (LDH) of heart of rats was either not sensitive enough to detect the effect of crude oil vapour or that the co-administration of vitamin E was able to modulate the effect of crude oil vapour on LDH
- The transferases (GGT, AST and ALT) of heart of rats were good biomarkers for monitoring the effect of vapour of crude oil on heart using rat models
- Serum metabolites (total protein, total cholesterols and triglycerides) levels were elevated in rats exposed to crude oil vapour
- Vitamin E played an important role in modulating the effect of crude oil vapour on these serum metabolites

Generally, oral administration of vitamin E could reduce the risk posed by exposure to vapour of crude oil by 50%.

The present study has presented a documented report on the modulating effect of oral administration of vitamin E on the vapour of crude oil; however, further studies are required for better understanding.

The followings are probable area for further study,

- The mechanism through which vapour of crude oil elicits biochemical changes should be investigated
- Effect of raising oral administration of vitamin E above therapeutic dose on crude oil vapour would provide more insight into its toxicology
- This study should be extended to employ antioxidant markers with a view to understanding and predicting the best chemo modulating agent to vapour of crude oil.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Statement of ethical approval

This study involving the use of rats has been conducted in strict accordance with ethical guidelines and regulations. All experimental procedures and protocols were approved by the FUPRE Animal Ethics Committee (Approval No. EMT/002/2023) to ensure the humane treatment and welfare of the animals involved. Every effort was made to minimize any potential discomfort or distress to the animals during the course of the research.

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