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Evaluation of toxic effect of oral co-administration of crude oil and vitamin C on antioxidant system of albino rats

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ABSTRACT: The present study investigated the effect of co-administration of crude oil and vitamin C on some antioxidant biomarkers of rats' cellular system. Rats were grouped into four (A, B, C, D) and treated orally with normal saline, 400 mg/kg bw vitamin C, 0.1 ml crude oil + 400 mg/kg bw vitamin C and 0.1ml crude oil respectively. Enzymic antioxidant assayed for in the kidney, liver, stomach and serum of rats are catalase (CAT) and superoxide dismutase (SOD), along with concentrations of reduced glutathione (GSH) and malondialdehyde (MDA). Kidney GSH of rats treated with normal saline is about 2 folds that of rats treated with 0.1 ml crude oil, kidney GSH of rats treated with 400mg/kg bw vitamin C is about 3 folds that of rats treated with 0.1 ml crude oil whereas the GSH of kidney of rats treated with both 400mg/kg bw vitamin C and 0.1 ml crude oil is about 2 folds that of rats treated with only 0.1 ml crude oil. Activities of SOD of tissues of rats treated with normal saline and those treated with 400mg/kg bw. Vitamin C are about 2 folds that of rats treated with 0.1 ml crude oil. CAT activities of tissues of rats treated with 0.1 ml crude oil are significantly lower relative to the rats treated with normal saline ($P<0.05$). MDA concentration of tissues of rats treated with 0.1 ml crude oil is significantly higher ($p<0.05$) relative to the rats treated with normal saline, 400 mg/kg bw. Vitamin C and 400 mg/kg bw. Vitamin C + 0.1 ml crude oil (A, B and C). MDA concentrations of kidney, liver and stomach of rats treated with 0.1 ml crude oil is significantly higher ($p<0.05$) than those of rats treated with both 400 gm/kg bw. Vitamin C and 0.1 ml crude oil. Particularly, serum MDA of rats treated with 0.1 ml crude oil about 2 folds that of rats treated with 400 mg/kg bw vitamin C + 0.1 ml crude oil. The present study generated data that suggest crude oil as the cause of oxidative stress in tissues of rats through a mechanism that depletes both enzymic and non-enzymic antioxidants while increasing the level of malondialdehyde.

Introduction

Nigeria is a major producer of crude oil and importance or of this commodity has been highly manifested in the nation's economy. Starting from the early 70's, the petroleum industry has become the dominant industry in the economy following quickly after the agriculture the dominant industry before the discovery of crude oil. It has dictated the pace of economic, political, social and cultural progress in the country (Adeyemi and Adeyemi, 2020a).

In the past few decades, there has been much concern about the adverse health effects of environmental contaminants in general and Crude Oil in particular around the Niger Delta region of Nigeria where all the crude Oil exploration is taking place. One of the major concern of these is it effect on human and his immediate environment (Sam *et* al., 2017; Ite*et al*., 2018)

Human and industrial activities such as drilling, manufacturing, storing, transporting, waste management of oil and vandalizing of oil pipelines in the petroleum sector result in extensive pollution

of the entire aquatic ecosystem with petroleum hydrocarbons (Zabbey*et al.*, 2017). These constitute serious socioeconomic and public health hazards. The Niger Delta region of Nigeria where oil is produced in large quantity is a purely agrarian society where the people depend on farming and fishing for survival (Nriagu *et al*., 2016). When the aquatic ecosystem is polluted with oil and petroleum hydrocarbons, these dangerous substances may accumulate in fish and other sea foods and farm produce from the adjoining farmlands and eventually get to man and animals probably through the food chain as observed by Adeyemi et al., (2016).

Crude oil is a mixture of a wide variety of constituents. It consists primarily of hydrocarbons of various molecular weights and other organic compounds. Crude oil also contains hundreds of substances that include benzene, chromium, iron, mercury, nickel, nitrogen, oxygen, sulfur, toluene, and xylene. Total petroleum hydrocarbon (TPH) is a term used to describe the several hundred chemical compounds that originally come from crude oil (Abdel-Shafy and Mansour, 2016). The main constituents of crude oil can be grouped into several broad classes of compounds: saturates (including waxes), aromatics, resins and asphaltenes. Saturates are alkanes with structures of C_nH_{2n+2} (aliphatics) or C_nH_{2n} in the case of cyclic saturates (alicyclics). Aromatics are compounds that have at least one benzene ring as part of their chemical structure. Resins and asphaltenes are similar in many ways such that asphaltenes can be thought of as large resins. Both groups are thought to be composed of condensed aromatic nuclei which may carry alkyl and alicyclic systems containing hetero-atoms such as nitrogen, sulphur and oxygen. Metals such as nickel, vanadium and iron are also associated with asphaltenes (Speight, 2014).

Crude oil kills plants and animals in the estuarine zone. It settles on beaches and kills organisms. It also settles on ocean floor and kills benthic (bottom-dwelling) organisms such as crabs. Oil poisons algae, disrupts major food chains and decreases the yield of edible crustaceans. It also coats birds, impairing their flight or reducing the insulating property of their feathers, thus making the birds more vulnerable to cold. Oil endangers fish hatcheries in coastal waters and as well contaminates the flesh of commercially valuable fish. The inhabitants of the oil-polluted areas, who are mostly fishermen, harvest the fish from crude oil-polluted water for human consumption (Siles and Margesin, 2018).

Furthermore, crude petroleum is seriously abused; it is being ingested as an antidote to poisons and as laxative in the Niger Delta (Becki, 2007). This is of great concern and has prompted the present study. Ingestion of crude oil for therapeutic purposes is a new narrative.

Ingestion of crude oil has been reported to induce oxidative damage through generation of free radicals leading to lipid peroxidation of crucial cellular macromolecules such as DNA, lipids and proteins (Aruoma, 1993, Adeyemi and Adeyemi, 2020b). Potent antioxidant such as Ascorbic acid (AA) is used as a therapeutic agent against many diseases and in the prevention of the adverse effects of stress factors in laboratory animals and humans. Vitamin C plays an important role in the body especially when the natural antioxidants are overwhelmed and exhausted, by donating a free molecule of hydrogen that detoxifies the harmful reactive oxygen species generated (Adeyemi and Adeyemi, 2020b). Vitamin C is also known to potentiate gamma-amino butyric acid (GABA) which reduces neurotransmission, including release of corticosteroids. This research evaluates the effect of vitamin C on cellular antioxidant system of rats treated with oral administration of crude oil.

Materials and Methods

Reagents

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

Crude oil

The crude oil (Nigerian Bonny Light (NBL) was obtained from Warri Refinery and Petrochemical Company (WRPC).

Experimental rats and treatments

Twenty adult Albino rats (*Rattus novergicus*) of mean weight 118±3.0 were obtained from the Animal Holding of the Department of Anatomy University of Benin, Benin-City, Nigeria. The rats were classified into 4 groups as follows;

Groups	Treatments
Group A	Control treated with normal saline orally over a period of 20 days
Group B	Treated with 400mg/kg bw Vit C. over a period of 20 days
Group C	Treated with 0.1 ml crude oil + 400mg/kg bw Vit C. over a period of 20 days
Group D	Treated with 0.1 ml crude oil over a period of 20 days

Ethical Clearance

All animals were housed under standard laboratory conditions with free access to water *ad-libitum* and balanced pellets food. The housing temperature was (25±1ºC) with 12h dark /light cycle and 50% humidity. All ethical guidelines on the use of animals for investigational purposes were followed and the experiment protocol was approved by Federal University of Petroleum Resources, Effurun (FUPRE), Nigeria ethics committee (FUPRE/ECC2019/SC/EMT001).

Tissue Homogenate and Serum

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 0.25M 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 -8ºC until required for use.

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 0.25M sucrose solution to the tune of 1 in 30 dilutions. The diluted homogenates were stored at temperature of -8ºC until required for use.

Biochemical Assays

The MDA concentration in the serum and tissues of rats experimental was determined following the method described by Bird *et al.* (1982). MDA, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. MDA reacts with thiobarbituric acid to give a red complex absorbing light at 535nm.

The GSH concentration in the tissues of experimental rats was determined following the method described by Jollow *et al,* (1974). The reduced form of glutathione comprises in most instances the bulk of cellular non protein sulfyhydryl group. The method is based upon the development of a relatively stable yellow colour when 5,5'- dithiobis (-2-nitrobenzoic acid) Ellman reagent is added to sulfyhydryl compound. The chromophoric product (yellow complex) resulting from the reaction of Ellman reagent with reduced glutathione, 2-nitro-5-thiobenzoic acid is proportional to the concentration of reduced glutathione.

The SOD activity of the tissues of experimental animals was determined following the method described by Misra and Fridovich (1972). The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 forms the basis for a simple assay of dismutase. O_2^- generated from xanthine oxidase reaction causes the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O^{2-} introduced increases with increasing pH, and with increasing concentration of epinephrine. These results led to the proposal that autoxidation of epinephrine proceeds by at least two distinct pathways, only one of which is free radical chain reaction involving superoxide radical (O_2^-) and hence inhibitable by SOD.

The catalase activity of the tissue homogenate obtained from the experimental animals was determined following the method described by Sinha (1971). In thismethod dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as an unstable intermediate. The absorbance value of the chromic acetate produced is read at 570-610nm on a spectrophotometer. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere with the colorimetric determination of chromic acetate.

Statistical Analysis

All numerical results were obtained from the four (4) groups (control and treated). Data obtained were presented as mean ± SEM and subjected to statistical analysis using a one way analysis of variance method of Steel and Torrie (1960). Significant difference between the treatment means was determined at 95%confidence level using Duncan's Multiple Range Test (Duncan, 1955).

Results

Table 1 shows the results of concentration of MDA of tissues of rats given oral administration of crude oil. MDA concentration of tissues of rats treated with normal saline was significantly lower ($p<0.05$) relative to the groups treated with vitamin C, crude oil + vitamin C and crude oil only (B, C and D). However, there was no significant difference ($p>0.05$) between serum MDA level of rats treated with normal saline and rats treated with vitamin C only. MDA concentrations of kidney, liver and stomach of rats treated with crude oil only was about 2 folds that of rats treated with normal saline. Serum MDA of rats treated with crude oil only was found to be about 4 folds that of rats treated with normal saline.

Table 1: Concentration of malondialdehyde (MDA) (nmol/L) in selected tissues of rats given oral administration of crude oil over a period of 20 days

Group	Kidney	Liver	Stomach	Serum
A	16.81 ± 1.00^{a}	13.21 ± 1.11^{a}	4.64 ± 0.88^{a}	1.09 ± 0.10^{a}
B	14.38 ± 0.95^{b}	10.88 ± 1.00^{b}	4.32 ± 0.76^{b}	1.06 ± 0.06^{a}
C	23.56 ± 1.56^{c}	16.94 ± 1.28^{c}	6.54 ± 1.00^{c}	2.00 ± 0.54^{b}
D	30.55 ± 2.11^{d}	24.10 ± 1.59^{d}	9.78 ± 1.12^{d}	4.10 ± 0.87^{c}

Tabulated results are means of 5 determinations ± SEM. Values carrying different notations are significantly different ($p<0.05$).

Table 2 presents the concentration of GSH in selected tissues of rats given oral administration of crude oil. Data revealed significant difference ($p<0.05$) among other tissues GSH of rats in rat groups treated with normal saline and vitamin C only, except the stomach. Kidney GSH of rats treated with normal saline is about 2 folds that of rats treated with crude oil only, while kidney GSH of rats treated with vitamin C only is about 3 folds that of rats treated with crude oil only. Noteworthy, kidney GSH of rats treated with crude oil+ vitamin C is significantly higher than that of rats treated with crude oil only. GSH of stomach of rats treated with normal saline and that of rats treated with vitamin C is about 3 folds that of rats treated with crude oil only, while GSH concentration of serum of treated with crude oil and vitamin C is 3 folds that of rats treated with crude oil only.

Table 2: Concentration of reduced glutathione (GSH) (mg/ml) in selected tissues of rats given oral administration of crude oil over a period of 20 days

Group	Kidney	Liver	Stomach	Serum
A	0.98 ± 0.05^{a}	1.00 ± 0.07^{a}	0.54 ± 0.02^{a}	0.36 ± 0.01^{a}
B	1.16 ± 0.06^{b}	1.24 ± 0.06^{b}	0.57 ± 0.02^{a}	0.42 ± 0.02^{b}
C	0.77 ± 0.03^{c}	0.87 ± 0.02^{c}	0.24 ± 0.02^{b}	0.30 ± 0.01^{c}
D	0.38 ± 0.02^{d}	0.44 ± 0.02^{d}	0.19 ± 0.01^{c}	0.10 ± 0.01^{d}

Tabulated results are means of 5 determinations ± SEM. Values carrying different notations are significantly different ($p<0.05$).

Specific activity of SOD of selected tissues of rats given oral administration of crude oil is presented in Table 3. Generally, there was no significant difference between the SOD activities of tissues of rats in treated with normal saline and those of rats treated with vitamin C only ($p<0.05$), however, SOD activities of these Groups of rats were significantly higher than those of the remaining groups of rats ($p<0.05$). SOD activity of tissues of rats treated with crude oil only, in particular, is about 50% that of rats treated with normal saline.

Generally, CAT activities of tissues of rats treated with crude oil only are significantly lower relative to the rats treated with normal saline ($P<0.05$) (Table 4). CAT activities of tissues of rats treated with normal saline and those treated with vitamin C only are not significantly different ($p>0.05$). CAT activity of tissues of rats treated with crude oil + vitamin C, however, is significantly lower relative to rats treated with vitamin C only and significantly higher than that of rats treated with crude oil only ($p<0.05$).

Table 3: Specific activity of superoxide dismutase (SOD) (U/mg protein) in selected tissues of rats given oral administration of crude oil over a period of 20 days

Group	Kidney	Liver	Stomach	Serum
A	1.11 ± 0.04^{a}	0.92 ± 0.03^{a}	0.49 ± 0.01^{a}	0.36 ± 0.02^{a}
B	1.10 ± 0.04^{a}	0.94 ± 0.04^{a}	0.50 ± 0.01^{a}	0.35 ± 0.01^{a}
C	0.74 ± 0.03^{b}	0.68 ± 0.02^{b}	0.36 ± 0.01^{b}	0.28 ± 0.01^{b}
D	0.45 ± 0.02^{c}	0.37 ± 0.01^{c}	0.22 ± 0.01^{c}	0.16 ± 0.01^{c}

Tabulated results are means of 5 determinations ± SEM. Values carrying different notations are significantly different ($p<0.05$).

Table 4: Specific activity of catalase (CAT) (µmole of H_2O_2 decomposed/min/mg protein) in selected tissues of rats given oral administration of crude oil over a period of 20 days

Group	Kidney	Liver	Stomach	Serum
A	1.10 ± 0.03^{a}	0.62 ± 0.02^{a}	0.32 ± 0.02^{a}	0.34 ± 0.02^{a}
B	1.10 ± 0.04^{a}	0.64 ± 0.03^{a}	0.33 ± 0.01^{a}	0.33 ± 0.02^{a}
C	0.82 ± 0.02^{b}	0.45 ± 0.02^{b}	0.22 ± 0.01^{b}	0.24 ± 0.02^{b}
D	0.51 ± 0.02^{c}	0.33 ± 0.01^{c}	0.14 ± 0.01^{c}	0.12 ± 0.01^{c}

Tabulated results are means of 5 determinations ± SEM. Values carrying different notations are significantly different ($p<0.05$).

Discussion

This study was spurred by the information that villagers along the crude oil impacted areas are so ignorant of the hazardous effect that they are in the habit of using crude oil as a therapeutic agent. More so, the study investigated administration of vitamin C alongside crude oil for possible chemopreventive action against the deleterious effect of ingested crude oil.

MDA concentrations of liver, kidney, stomach and serum of rats treated with crude oil only is significantly higher ($p<0.05$) that those of rats treated with crude oil + vitamin C (Table 1). These data suggests the presence of reactive oxygen species which might have arisen as a result of ingestion of crude oil. Co-administration of vitamin C with crude oil was able to reduce the level of MDA. Previous study revealed that reactive oxygen species (ROS) have been implicated in the development of cardiovascular and cerebrovascular diseases by causing membrane lipid peroxides and toxic malondialdehyde (MDA) which can damage proteins and DNA and have also been implicated in carcinogenesis (Adeyemi, 2015). Oxidation of lipids modifies membranes and impairs their function. Fluidity is decreased, membrane bound enzymes and receptors are inactivated, red blood cells are damaged and endothelial cells are injured, increasing blood vessels fragility (Adeyemi and Isukuru, 2017). Increased MDA level in the tissues of rats (Table 1) could be due to generation of ROS following ingestion of crude oil.

Naturally, non-enzymic antioxidants such as GSH and enzymic antioxidants such as SOD and catalase are present in tissues of animals and plants, and they are depleted during oxidative damage (Adeyemi and Adeyemi, 2020b). GSH, for instance, is a tripeptide and an antioxidant that is important in detoxification of endogenously generated peroxides and exogenous chemical compounds. Depletion of GSH concentration in tissues decreases cellular antioxidant defence ability and increase oxidative stress (Adeyemi and Adeyemi, 2020c). The GSH of tissues of rats treated with crude oil only is significantly lower than those of rats treated with crude oil + vitamin C ($p<0.01$) (Table 2). This observation portends that vitamin C, perhaps, ameliorates the effect of the crude oil.

Table 3 revealed that ingestion of crude oil inactivated and/or decreased the activity of SOD of tissues of rats treated with it while co-administration of vitamin C with crude oil activated and/or increased SOD activity of the tissues of rats. Report has shown that, in dismutation reaction in which hydrogen peroxide is generated, SOD selectively eliminates superoxide radicals thereby causing a decrease in SOD activity. Reduction of SOD activity also may be due to an inhibited biosynthesis of enzyme molecules by environmental pollutants or its metabolites and/or to the effect of hydrogen peroxide, which may directly alter its activity (Adeyemi, 2015). Data from this study present strong evidence that crude oil generates metabolites that could overwhelm SOD protective ability while co-administration of vitamin C could reduce the oxidative effect by half (Table 3).

Catalase (CAT) is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Adeyemi, 2015). It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). As seen in Table 4, CAT activities of tissues of rats treated with crude oil and vitamin C are significantly higher than those of tissues of rats treated with crude oil only ($p<0.05$). This data lend credence to the submission that Vitamin C is a candidate for boosting the antioxidant mechanisms of the body against crude oil.

Conclusion

The present study shows data suggesting that crude oil causes oxidative stress in the tissues of rats depleting both enzymic and non-enzymic antioxidants, and increasing the level of malondialdehyde. The study also presents evidence suggesting that vitamin C could serve a chemopreventive role against the condition of oxidative stress induced by crude oil when co-administered. Experimental evidence from this study is neither for nor against use of crude oil for therapeutic purposes but rather suggesting inclusion of vitamin C or vitamin C rich food in the diet of people living in crude oil impacted areas.

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