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Effects of Selenium Fortified Diet on Inflammatory Markers in Wistar Albino Rats Exposed to Crude Oil

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Abstract
A number of studies have shown that crude oil and related chemicals are harmful to the body. As a result of this, the means of mitigating these harmful effects needs to be investigated. Hence, this study was designed to investigate the ameliorative role of selenium as a potential antidote for crude oil induced toxicity. Forty male albino rats were disseminated into groups A and B. Animals in group A was fed with normal diet for one month while those in group B were fed with crude oil adulterated diet (COAD) for the same duration as those in group A. Six rats from each group was used to determine liver function enzyme activity, so as to establish the hepatotoxicity of crude oil adulterated diet on the liver of the exposed animals. Thereafter, rats in group B were allocated into three groups (B1, B2 and B3) of six rats each. All the rats were then given normal diets except those in group B2 and B3 whose diets were supplemented with sodium selenite of 0.5µg Se/g diet and 1.0µg Se/g diet respectively. The rats were exposed to these diet and water for three months. At the end of this period, inflammatory markers parameters (erythrocyte sedimentation rate, C-reactive protein, ceruloplasmin and creatinine), antioxidants enzymes (superoxide dismutase (SOD), Catalase, MnSOD, Cu/ZnSOD), lipid peroxidation, lipid profile markers and liver function enzymes were estimated. The results indicated that treatment of diet with selenium modulated the crude oil-induced inflammation. It is of note therefore, that selenium has a promising role and it is worth being considered as a potent remedy against crude oil-induced biochemical toxicity.

Key words: Crude oil, Diet, Inflammation, Liver, Selenium

Introduction
Crude oil, sometimes called petroleum is the un-processed naturally occurring oil found beneath the earth crust. (Igwe *et al.,* 2017). The main constituents of crude oil are saturates (including waxes), aromatics, resins and asphaltenes. Saturates are alkanes also known as aliphaticsor the cyclic saturates called alicyclics. (Narve*et al*., 2001).

Crude oil was discovered in Nigeria in 1956 and the activities involved in its extraction, processing and uses have led to the release of crude oil, its refined products and their chemical constituents such as aliphatic and polycyclic aromatic hydrocarbons into the environment (Otitoju and Onwurah, 2007; Ovuru and Ekweozor, 2004; Medubari and Elijah, 2014).Crude oil spill is a major source of environmental pollution by petroleum which constitutes danger to both aquatic and terrestrial animals (Medubari and Elijah, 2014).

Petroleum hydrocarbons get into animals through various forms. The ingestion of crude oil or its byproducts are some of the ways animals are exposed to petroleum hydrocarbon toxicity (Achuba and Awhin, 2009; Okpoghono et al., 2018a). Petroleum has been associated with induction oxidative stress (Achuba, 2010). Akpofure *et al*. (2000) reported that acute exposure to crude oil either through breathing or ingestion usually causes difficulty in breathing, confusion, nausea, headaches and other central nervous system effects. Also, Jude *et al*. (2014), suggested that crude oil exposure even in low concentration has the potential to affect haematological, biochemical parameters and can result in renal diseases.

The toxicity of myriads of hydrocarbons to living organisms has been attributed to its complex chemical composition (Okpoghono et al., 2018b).When crude oil enters the environments, it undergoes both physical and chemical transformations as well as microbial degradation to form more harmful substances (Bhatia, 2001). Zakrzewski (2002), reported that these products contains carcinogenic compounds that affect humans and animals living in crude oil polluted environments. The effect of crude oil toxicity can easily be manifested on various organs like the skin, lungs, liver and kidney (Okoye *et al*., 2014;Ita*et al*., 2014). Most crude oil compounds are lipophilic (quality of dissolving in lipids) in nature and have the ability to exert biochemical, physiological and cellular damages in animals

(Orisakwe *et al*., 2004;Braide *et al*., 2011). Also, Otaigbe and Adesina (2005) reported that direct exposure to crude oil lead to burning sensation, irritation, erythema (abnormal redness and inflammation of the skin), followed by formation of vesicles, blisters and extensive epidermolysis (connective tissue disease).

A variety of plants and inorganic products have been used to reduce and manage the clinical implication of crude oil in experimental animals and polluted environments. One technique used to recover polluted area is Bioremediation which has a very high efficiency rate and low cost (Bidoia *et al*., 2010). This technique is an eco-friendly procedure that utilizes naturally occurring microorganisms which transform toxic substances to non-toxic compounds and its being used for the treatment of soil and ground water contamination. (Milic *et al*., 2009).Achuba and Otuya (2006) and Uboh *et al* (2009) reported the prevention of the toxic effect of hydrocarbons by vitamins.Similarly, different plant- derived substances and beef live rreduced hepatotoxicity and haematoxicity of diesel (Ujowundu *et al*, 2012; Achuba and Ogwumu, 2014a). Furthermore, the architectural integrity of the hepatic and renal cells of rats was preserved by palm oil treatment of diesel-tainted diet (Achuba and Ogwumu, 2014b). Similarly, Achuba and Nwokogba (2015), reported the protective ability of natural honey on hydrocarbon- induced biochemical indices. Recently, Ita *et al*, (2016) reported also the protection of Vitamins C and E ameliorated crude oil-induced immune function interference.

With the use of plants and their products to manage crude oil toxicity, researchers are now shifting their attention to mineral metals. One of such minerals with a myriad of health giving properties is Selenium (Se).

Since its discovery in 1818 by Jacob Berzelius, the importance of selenium cannot be over-emphasized. Selenium is an essential naturally occurring trace element that is used for a wide variety of biological processes in mammals. (Schomburg *et al*., 2004). When selenium is incorporated with proteins, selenoproteins are formed which actas antioxidant enzymes that help to prevent cellular damage from free radicals and the development of chronic diseases such as cancer and heart disease. (Combs and Gray, 1998). So far, twenty five (25) selenoproteins has been identified in humans (Papp *et a*l., 2007) and the families of glutathione peroxidases (GPxs) and thioredoxinreductases (TrxRs) are the ones that participate in antioxidant defence. (Kryukov*et al*., 2003). Selenium has also been shown to prevent cisplatin hepatotoxicity in mice (Liao *et al*., 2008). Jihen *et al*, (2008), reported that selenium has a cooperative effect in the protection against cadmium-induced structural damage in liver of rat. Also, in a study conducted by Davis *et al*, (2012), they showed that different selenoproteins are involved in the prevention against cancer. Meta-analytic studies of the literature showed that selenium deficiency was a cancer promoting factor. Furthermore, in a report by Saricaoglu *et al*, (2005), it was observed that selenium plays an important role in the treatment of inorganic mercury intoxication.

The main route for selenium intake into the body is via food (Rayman, 2008), although the amount of selenium varies according to food type. Foods like cereal, meat, eggs, and milk/dairy products have been shown to contain significant amounts of selenium (Rayman, 2008).

Studies on the pathology of crude oil on exposed animals and the search for ameliorating agent against its toxicity are on-going. However, no research has been carried out to investigate the ability of Selenium to mitigate the clinical consequences of crude oil on exposed animals. Therefore, this study will be carried out to determine the possible ameliorative effect of Selenium against crude oil induced toxicity in experimental rats.

Materials and Methods

Materials

Crude oil was obtained from the Department of Petroleum Resources, Port Harcourt, Nigeria. High quality analytical grade chemicals were used.

Experimental Animals

The rats used for this investigation were fifty male albino Wistar rats. The rats were got from Animal House of Faculty of Basic Medical Sciences, Delta State,University, Abraka, Nigeria. The rats weighed between 165 g and 170 gand wereacclimatized on grower's mash and experimental conditions for seven days in clean plastic cages.

Treatment of Animals

The fifty male albino Wistar rats were at first divided into two groups (A and B) of fifteen and thirty five rats, respectively. Rats in group A was fed with normal diet for one month while those in group B was fed with crude oil adulterated diet (COAD) at a concentration of 4ml/100g of feed for the same duration as those in group A.Thereafter, six rats from each group were used to determine liver function enzymes activity, so as to establish the clinical implication of crude oil adulterated diet on the animals. After the preliminary study, rats in group B were further divided into three groups of six rats each (B1, B2 and B3). All the rats were then given normal diets except those in group B2 and B3 whose diets were supplemented with sodium selenite of 0.5µgSe/gdiet and

1.0µgSe/gdiet, respectively. The rats had water adlibitum and these diets for three months.

Collection of Blood Samples

After three months of treatment, the rats were sacrificed after being anaesthetized with chloroform and blood collected by cardiac puncture into containers. Blood sample (10ml) was taken into plain sample bottles and left to stand for 2hours to allow for clotting. The clotted blood was centrifuged at 4000rpm for 10 minutes and the serum collected with Pasteur pipette into sample bottles. The collected serum was preserved at −20˚C until needed for assay.

Determination of Haematological and Biochemical Indices

ESR was estimated using the method of Westegren(1957) and the latex agglutination method was used to estimate serum C-reactive protein (C-RP) levels (Singer, 1957). Ceruloplasmin concentration was estimated according to Sunderman and Nomoto (1970) method while creatinine concentration was estimated according to the method of Lustgarten and Wenk (1972). The activities of liver function enzymes(AST and ALT) and lipid profiles were determined using standard kits (Randox, UK). A breakdown product of lipid peroxidation thiobarbituric acid reactive substance (TBARS) was measured in the tissue homogenates according to Gutteridge and Wilkins (1982). The activity of superoxide dismutases were estimated according to Misra and Fredorich (1972) and Crapo et al (1978). Catalase activity was estimated by Cohen *et al*., (1970) method.

Results

The result shows that there was no significant ($p<0.05$) difference in the feeds consumed by rats in all groups (Table 1). The ingestion of selenium enriched diet improved crude oil tainted diet stimulated weight loss significantly ($p<0.05$); with a more increase noticeable in group fed with 1.0µg Se (Fig 1).Levels of serum transferases that rose due to crude oil diet consumption decreased close to control value on treatment with selenium, with a more significant difference noticed in the group treated with 0.5µ/g Se (Fig 2). In a similar vein, intake of crude oil adulterated diet culminated in negative alteration of lipid panel comparable to the control group. Treatment with selenium significantly ($p<0.05$) change these values close to control, with more effect noticed in group treated with 0.5µg Se (Fig 3). Also, elevated levels of ESR, C-RP and creatinine in group fed with crude oil adulterated diet was mitigated by selenium (Fig 4). Treatment of diet with selenium significantly ($p<0.05$) decreased ceruloplasmin close to control value(Fig 5). Serum antioxidant enzymes activities were decreased by intake of crude oil when compared to control values. Treatment with selenium however, significantly ($p<0.05$) increase, even more than control values (Fig 6). Figure 7 showed a significant ($p<0.05$) decrease in lipid peroxidation on feeding the rats with feed enriched with selenium.

Table 1: Feed intake (100g/week) of experimental rats.

Parameter	Control	COD	COD + 0.5µg Se	COD + 1.0µg Se
Feed	233±12.0[a]	201±49.8[b]	227±2.94[a]	228±4.39[a]

Results are expressed as mean ± SD with n=6. All values sharing similar alphabetical superscripts indicates a non-significant difference at $p<0.05$.

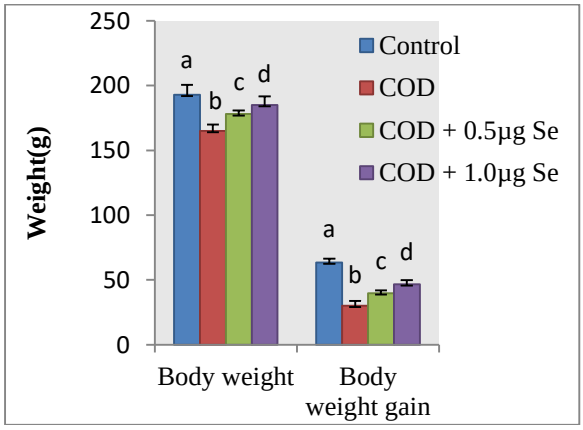

Figure 1: Body weight and weight gain of experimental rats at end of exposure period. Bars represent mean values of groups, with n =6. Different superscript indicates significant at $p<0.05$.

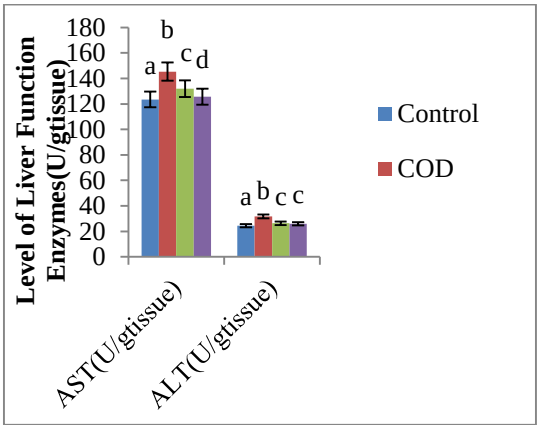

Figure 2: Effect of selenium treated diet on AST and ALT Activities of rats exposed to crude oil. Bars represent mean values of groups, with n = 6. Different superscript indicates significant at $p<0.05$

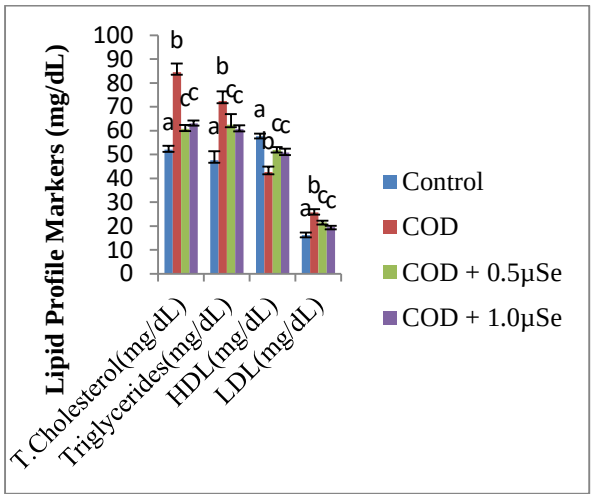

Figure 3: Effect of selenium treated diet on lipid profile markers of rats exposed to crude oil. Bars represent mean values of groups, with n = 6. Different superscript indicates significant at $p<0.05$.

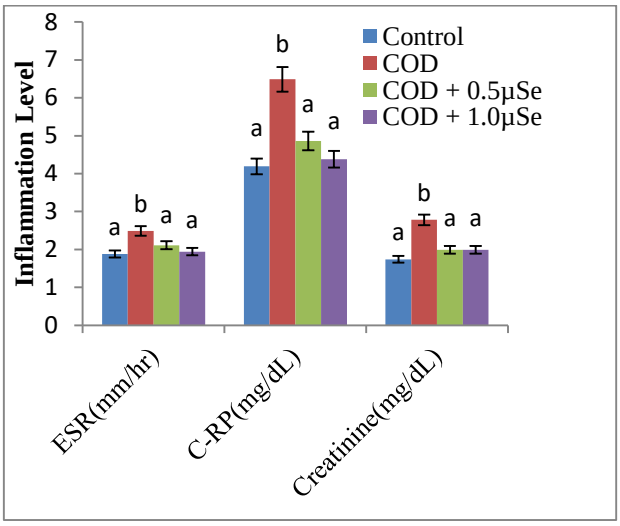

Figure 4: Effect of selenium on some inflammatory markers of rats exposed to crude oil. Bars represent mean values, with n =6.Different superscript indicates significant at $p<0.05$.

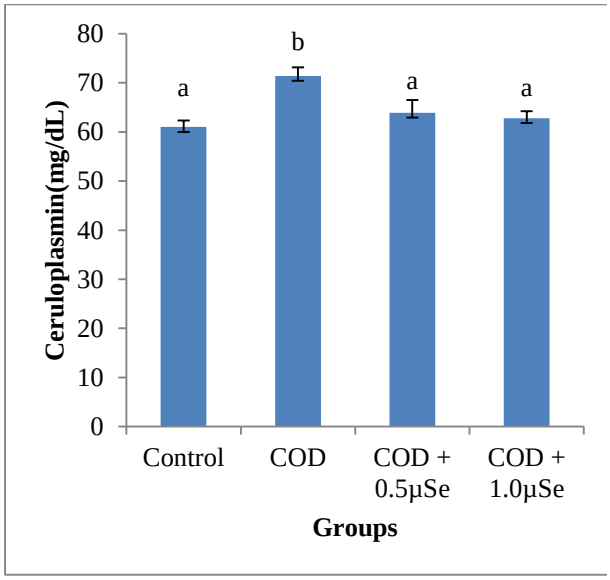

Figure 5: Activity of selenium on ceruloplasmin level on rats exposed to crude oil. Bars represent mean values of groups, with n = 6. Different superscript indicates significant at $p<0.05$.

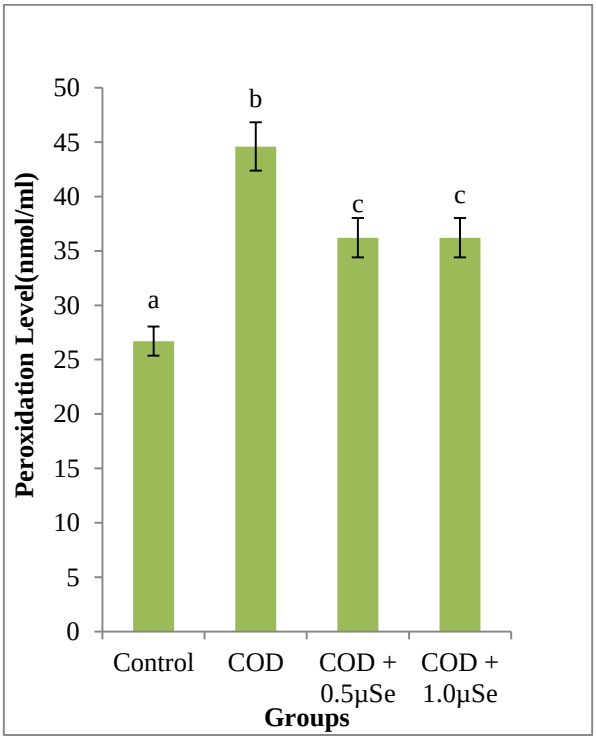

Figure 6: Effect of selenium on lipid peroxidation levels of rats exposed to crude oil. Bars represent mean values with n = 6. Different superscript indicates significant at $p<0.05$.

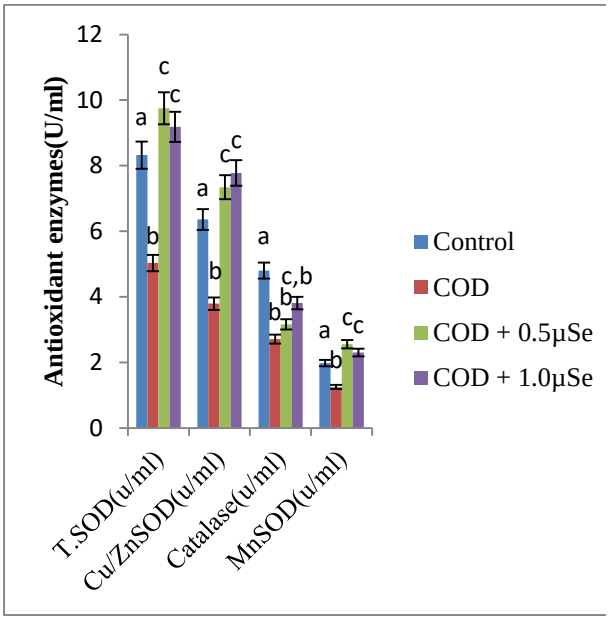

Figure 7**:** Effect of selenium on some antioxidant enzymes in serum of rats exposed to crude oil. Bars represent mean values, with n = 6. Different superscript indicates significant at $p<0.05$

Discussion

Hydrocarbon adulterated diet is linked with a variety of pathophysiological imports in animals (Achuba, 2018). This informs the search for potential antidote for petroleum toxicity on animals and man. In this study, it is obvious that selenium supplementation of diet did not affect the feed intake (Table 1). This is indicated by the non-significant difference among all the treated groups. However, the weight gain of rats previously exposed to petroleum contaminated diet when compared to rats fed with normal diet was brought close to control values by selenium supplementation of diet (Fig. 1). This implies that supplementation of diet with selenium improved the health status of petroleum exposed animals. Previous report indicated that weight gain is a measure of health status of an animal (Fadairo and Otite-Douglas 2015; Ezedom and Asagba, 2016).

Also, supplementation of diet with selenium improved crude oil induced liver damage (Fig. 2). This is evidenced by the restoration of liver enzyme activities close to the values in control rats. This observation is no surprise because crude oil mediates in free radical generation (Achuba and Osakwe. 2003; Nwaogu and Onyeze, 2014). In the same vein, the antioxidative potency of substances against crude oil toxicity is severally reported (Achuba and Otuya, 2006; Achuba and Ahwin, 2009; Uboh et al., 2009; Ita *et al.*, 2016).This may be the reason why selenium supplementation of diet attenuated crude oil induced liver damage.

Consumption of crude oil adulterated diet altered the lipid panel of rats (Fig. 3). The observed increase in total cholesterol, triglycerides, LDL cholesterol and decrease in HDL cholesterol values shows an import of injury or damage to cell membrane or loss effectiveness and integrity in regulating lipid metabolism (Uboh *et al*., 2005; Ogara *et al*., 2016). However, treatment with selenium supplemented diet modulated these values close to control values (Fig. 3). This is in line with Imen *et al*., (2017), which reported that selenium acts as an antioxidant and inhibits the oxidative processes of lipids and lipoproteins in cell membranes.

Erythrocyte sedimentation rate, C-reactive protein, ceruloplamine and creatinine have been reported as a measure of inflammation in animals (Guha *et al*., 2014). That selenium exhibited health promoting features is expressed in the positive alterations of petroleum-induced measures of inflammatory markers (Fig. 4). Petroleum hydrocarbon stimulated increase in some of these markers of inflammation has been documented (Achuba and Ogwumu, 2014a; Ita *et al*., 2014). Similarly, like selenium, vitamins C and E as well as honey have been reported to reverse these inflammatory markers in hydrocarbon intoxicated rats (Ita *et al*., 2014). The anti- inflammatory disposition of selenium is no surprise since one of the mechanisms behind the clinical implication of petroleum hydrocarbon is the induction of free radicals (Achuba, 2010), which is quenched by the antioxidant potentials of selenium.

The relationship between lipid peroxidation, enzymatic antioxidants and disease progression has been documented (Pham-huy et al., 2008).like the alteration in lipid panel, crude oil intake culminated in enhanced lipid peroxidation (Fig.6) and decreased antioxidant enzymes activities(Fig.7).This is an indication of oxidative stress or damage which is in agreement with previous studies (Achuba, 2010). Oxidative stress has been reported to play a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases (Haidara *et al*., 2006). However, supplementation of diet with selenium decreased serum lipid peroxidation product (MDA) (Fig. 6) as well as increased the activities of antioxidant enzymes (total SOD, catalase, MnSOD and Cu/ZnSOD) (Fig. 7). This is in line with previous studies which recognized selenium as a trace element of great importance for human health which protects the cells from the harmful effects of free radicals attack (Danesi, *et al* ., 2006; El-Demerdash, 2004).

This work shows that crude oil negatively affect the physiological as well as biochemical status of rats and also the ability of selenium to reverse these negative alterations due to its antioxidant and anti-inflammatory abilities. This study has thus

demonstrated that crude oil induced inflammation/oxidative stress can be mitigated by selenium treated diet.

This study has established that selenium can protect against crude oil induced biochemical toxicity/inflammation as clearly evident by its role in reversing these biochemical alterations to near control values. The ability of selenium to reverse these alterations is clearly as a result of its antioxidants and anti-inflammatory ability. It is of note therefore, that selenium has a promising role and it is worth being considered as a potent remedy against crude oil-induced biochemical toxicity.

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