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Crude Oil Induced Oxidative Changes and Chemoprotective Roles of *Ocimum gratissimum* and *Gongronema latifolium* Formulated Diet

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ABSTRACT

The antioxidant and hepatoprotective effects of *Ocimum gratissimum* and *Gongronema latifolium* formulated diet were evaluated in crude petroleum oil induced toxicity in rats. Exposure to crude oil caused significant ($p<0.05$) decrease in superoxide dismutase, glutathione peroxidase, reduced glutathione and increase malondialdehyde. Hepatocyte damage was indicated by the rise in aspartate aminotransferase, total bilirubin and fluctuations in other liver parameters. However, the group intoxicated and fed simultaneously with the formulated diet showed some level of stability and modification in the evaluated biochemical parameters, indicating potential therapeutic and protective role of *O. gratissimum* and *G. latifolium*. Thyroid hormones and lipid profile were also determined.

Indexing terms/Keywords

Crude oil, *Ocimum gratissimum*, *Gongronema latifolium,* oxidative damage, free radicals, antioxidants.

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INTRODUCTION

Crude petroleum oil is a major revenue contributor to the Nigerian economy. However, its exploration and exploitation pollutes the environment, presenting potential hazard to both aquatic and terrestrial species (Shore and Douben, 1994: Ujowundu et al., 2011). Crude oil is a complex mixture of hundreds of different hydrocarbons and chemicals (such as metals) which varies between geologic formations (Edwards, 1989; Coppock, 1995). Current public health trends have shown that petroleum oil company staff and other individuals exposed to crude oil might have an increased incidence of organ damage. The biochemical disposition of crude oil shows that, after absorption via pulmonary or gastrointestinal routes, it is transported in plasma initially bound to albumin and other larger proteins to the liver (Orisakwe et al.,2004).

Consuming plant products such as fruits, vegetables and spices can help overcome and/or ameliorate the toxic effects of crude oil. It is estimated that up to 2.7 million lives could potentially be saved each year if consumption of fruits and vegetables are sufficiently increased (WHO, 2002). Africa, and in particular Nigeria, is blessed with vast number of medicinal plants, spices, fruits and vegetables. However, they have remained under-utilized due to lack of awareness and popularization of technologies for utilization (Sheela, 2004). Plant based diet offers diverse group of bioactive (nutritional and phytochemical) compounds, playing important part in maintaining general good health (Chaturved et al., 2013). These bioactive compounds possess antioxidative potentials, which at low concentration compared to those of an oxidizable substrate (Halliwell and Gutterridge, 1989), significantly delay or prevent the oxidation of that substrate. They are capable of preventing or attenuating damages such as lipid peroxidation, oxidative damage to membrane, glycation of proteins and inactivation of enzymes caused by free radicals (Hamzah et al., 2013).

Ocimium gratissimum linn is a culinary herb indigenous to Nigeria with a pungent sweet smell and of the family Lamiaceae (Calixto, 2000). It is commonly called '*Nchanwu*' by Igbos in Nigeria and is used in cooking soups. The medicinal values of this plant lie in their phytochemicals - flavonoids, steroids, terpenoids, tannins and cardiac glycosides (Afolabi et al., 2007: Ujowundu et al., 2011), which produce definite physiological actions. In the same vein, *Gongronema latifolium* is a perennial climber crop belonging to Asclepiadaceae family. The origin of the plant is traced to Nigeria and it is called '*Utazi*' by Igbos in South-Easthern Nigeria (Hamzah et al., 2013). It is atropical rain forest plant primarily used as spice and vegetable and is rich in alkaloids, tannins, glycosides, polyphenols, saponins and flavonoids (Morebise et al., 2002; Atagho et al., 2009).

For years the local population has being victims of crude petroleum oil pollution. This could be via inhalation or ingestion via water, due to regular spillage incidents into water bodies and estuaries which serve as sources of water for domestic uses. Also, the uses of crude oil by indigenous or local dwellers as concoctions for the treatment various ailments such as gastrointestinal disorders, burns, foot rot and leg ulcers, poisoning and witchcraft (Orisakwe et al.,2004). The impacts of crude oil exposure to living systems need to be assessed and the toxicity determined. This study evaluated the protective effect of the combined use of indigenous vegetables- *O. gratissimum* and *G. latifolium* against oxidant generated by the cellular metabolism of chemical constituents of crude oil.

2. MATERIALS AND METHODS

2.1. Procurement and Preparation of Animal and Plant Materials

Male Albino Wistar rats were obtained from the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State of Nigeria. The crude oil was obtained from the quality control unit, Nigerian National Petroleum Corperation, Portharcourt, Nigeria. Rat pellets was purchased from vital feeds Nigeria and the indigenous spices (*O. gratissimum* and *G. latifolium*) were purchased from Orie Ukwu Market in Umuoshi Umunama Ezinihitte Mbaise, Nigeria. The fresh leaves of *G. latifolium* and *O. gratissimum* were air dried at room temperature, ground into powder and sieved through a micro pore sieve. The feed was formulated with 10 % *G. latifolium,* 10 % *O. gratissimum* and 80 % rat pellets. This study was approved by the ethics committee of the Department of Biochemistry, Federal University of technology, Owerri, Nigeria. The animals were handled in accordance with the guidelines on the care and well being of research animals (NIH, 1985).

2.2. Study Design

Preliminary toxicity test to determine the volume of crude oil that could cause toxicity was carried out as described by (Ujowundu et al., 2012). The eighteen rats (156-176 g) used in this study were maintained under standard conditions (temperature of 20 - 24 °C, light/darkness cycles of 12 hours). The rats were grouped into three, with six rats per group. Group I (control) were allowed the rat pellets only. Group II (formulated diet group) were challenged orally with 6 ml/kg body weight of crude oil and allowed the formulated diet freely. Group III (untreated group) were challenged orally with 6 ml/kg body weight of crude oil only. Water was provided *ad libitium* to all groups.

2.3. Serum and Liver Sample Preparation

The animals were sacrificed on the eight day after 24 hours fast. Blood samples were collected by cardiac puncture and the liver was removed and refrigerated. The serum prepared from the blood was used for the assay of lipid profile, liver parameters and thyroid hormones. Liver tissues of rats were excised, weighed and some part homogenized in potassium chloride (10 mM) phosphate buffer (1.15%) with Ethylenediamine tetra-acetic acid (EDTA; pH 7.4) and centrifuged at 12,000 x g for 60 minutes. The supernatant was used to assay for some oxidative stress parameters.

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2.4. Assessment of oxidative stress

Liver homogenate was used for the determination of catalase (CAT) (Aebi, 1984), superoxide dismutase (SOD) (Xin et al., 1991) and glutathione peroxidase (GPx) (Paglia and Valentine, 1967) activities. Also, reduced glutathione (GSH) (King and Wootton, 1959) and Malondialdehyde (MDA) in the liver homogenate was determined by the reaction with thiobarbituric acid (TBA) and used as a lipid peroxidation marker (Wallin et al., 1993).

2.5. Determination of Liver Function Markers

The quantitative *in-vitro* determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total protein (TP) and bilirubin in serum were measured as specified by commercial kit manufacturer - Randox laboratory ltd (Antrim, UK, BT29 4QY). Globulin was calculated thus: serum globulin = total protein – serum albumin (TP-ALB).

2.6. Lipid Profile Determination

Serum lipids – cholesterol, triglycerides, high density lipoprotein (HDL) – cholesterol, were quantified by the *in-vitro* determination of lipid concentration in serum as specified in the test kit by Randox laboratory ltd (Antrim, UK, BT29 4QY). Serum low density lipoprotein (LDL) - cholesterol and very low density lipoprotein (VLDL) - cholesterol were determined thus: VLDL (mmol/l) = Triglyceride /2.2; LDL (mMol/l) = total cholesterol– triglycerides/2.2 – HDL.

2.7. Determination of Serum of Thyroid Hormones

Triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) were determined using Strepta vidin-biotin kit (Biotron diagnostics Inc. Hemet CA USA). This is an ELISA method for the quantitative *in-vitro* determination based on the principle of solid phase enzyme-linked immunosorbent assay (Utiger, 1974; Skelley et al., 1973).

2.8. Data Analysis

Results were expressed as mean ± standard deviation and all data were subjected to analysis of variance (ANOVA). Significant differences between the treatment means were detected at 5% confidence level.

3. RESULTS AND DISCUSSION

After being taken up by an organism, hydrocarbons and their metabolites can enhance the production of reactive oxygen species (ROS) by several mechanisms that lead to cellular damage (Altenburger, 2003). ROS has been implicated in many human degenerative diseases, such as Alzheimer, cancer, diabetes etc (Hristozov, 2001; Orrenius, 2007). The effect of crude oil toxicity and treatments on oxidative stress enzymes and molecules is presented in Table1. Significant variations were observed in all the parameters (SOD, GPx, GSH and MDA) measured except in the activities of catalase. Exposure to crude oil induced significant depletion in the concentration reduced glutathione (GSH) and its metabolizing enzyme glutathione peroxidase (GPx). GSH is an endogenous antioxidant and its depletion is indicative of xenobiotics attack. The significant restoration of the depleted GSH and GPx in the formulated diet group is suggestive of the hepatoprotective ability of *O. gratissimum* and *G. latifolium* diet. Most xenobiotics induce formation of pro-oxidants and a relative decrease in antioxidants status of cells (Anon et al., 1992; Singh and Handa, 1995). This supports the observed significant decrease in SOD in the crude oil group. CAT, SOD and GPx are crucial in protecting cells against oxidative stress and damage. The observed significant decrease (except CAT) in the activities of these enzymes could be due to their involvement in antioxidative functions. Similarly, the increase in the concentration of lipid peroxidation product (MDA) confirms the induction of oxidative stress in rats exposed to crude oil without concomitant treatment with the vegetable diet (Achuba and Osakwe, 2003; Altenburger, 2003; Ujowundu et al., 2011; Mahmoud et al., 2011; Ujowundu et al., 2012).

Table 1: The effect of crude oil exposure and treatments on oxidative stress parameters

Groups	CAT (IU/g tissue)	SOD (IU/g tissue)	GSH (mg/g tissue)	GPx (IU/g tissue)	MDA (%TBARS)
Control	7.81 ± 0.37^{a}	1.11 ± 0.34^{b}	2.03 ± 0.08^{b}	531.09 ± 21.43^{c}	5.10 ± 1.05^{a}
Formulated Diet	7.62 ± 0.24^{a}	1.44 ± 0.32^{b}	2.13 ± 0.40^{b}	356.91 ± 13.60^{b}	4.75 ± 0.61^{a}
Crude oil	7.56 ± 0.44^{a}	0.37 ± 0.23^{a}	1.17 ± 0.09^{a}	318.81 ± 16.24^{a}	7.08 ± 0.80^{b}
F-value	0.42	9.91	14.00	126.94	6.75
P-value	0.68	0.013	0.005	0.000	0.029

Values (mean±SD of triplicate determinations) with different superscript on same column are significantly ($p<0.05$) diferrent

When hepatocytes membranes are allowed to be damaged by highly reactive free radical, as observed by the significant variation in the oxidative parameters in this study, a variety of liver transaminases are released into blood from the cytosol (Mitra et al., 1998). This indicates hepatocytes damage even without evident hepatic impairment. The results of liver function parameters presented in Table 2, shows a raised activity of AST and ALT in the crude oil exposed rats compared to control. This could be attributed to damage to the structural integrity of liver (Rosen and Keefe, 2000; Friedman et al., 2003; Ujowundu et al., 2011) and possible necrotic lesions in the hepatocytes (Ujowundu et al., 2011). However, the

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decreased AST and ALT activities in the formulated diet group affirms the protective potential of the two plants on hepatocytes. This corroborated with the works of Ujowundu et al. (2011; 2012) which reported the hepatoprotective and antioxidant potentials of *O. gratissimum* and *G. latifolium* respectively against petroleum based products-induced hepatotoxicity in albino rats. The antioxidants and other constituents of *O.gratissimum* and *G. latifolium* (Morebise et al., 2002; Afolabi et al.,2007; Atagho et al., 2009; Ujowundu et al., 2011) may have initiated the healing and regeneration of liver parenchyma and cells respectively (Thabrew et al., 1987).

Table 2: Effect of exposure to crude oil and treatment with *O.gratissimum* and *G. latifolium* on liver parameter

Groups	AST(IU/L)	ALT(IU/L)	Albumin(g/l)	TProtein(g/l)	Globulin(g/l)	Tbilirubin(g/l)
Control	42.33±8.96^{a}	18.97±1.34^{a}	43.66±3.21^{a}	95.33±4.70^{b}	52.00±5.29^{b}	10.33±.91^{a}
Formulated Diet	56.00±6.80^{a}	28.73±5.49^{b}	34.67±9.29^{a}	76.00±2.87^{a}	41.33±6.81^{b}	18.90±1.56^{b}
Crude oil	84.67±5.67^{b}	39.27±5.09^{c}	39.66±1.53^{a}	69.33±3.53^{a}	27.00±3.61^{a}	15.20±2.72^{b}
F-value	28.07	16.06	1.85	12.75	16.22	15.58
P-value	0.001	0.04	0.237	0.007	0.004	0.004

Values (mean±SD of triplicate determinations) with different superscript on same column are significantly (p<0.05) diferrent

Also, the decrease in total protein and globulin in the crude oil group may be attributed to the decrease in synthetic function of liver due to crude oil exposure. However, the restoration of total protein and albumin concentrations towards the control value in the formulated diet group indicates ameliorative effects of the vegetables. This agrees with the studies (Etim et al., 2008; George et al., 2012), which recorded a hepatoprotective potential of *O. gratissimum* and *G. latifolium* against ethanol-induced and CCl_4-induced hepatoxicity in albino rats respectively. Also, the significantly increased total bilirubin in crude oil and formulated diet group suggests increased haemolysis, which overwhelmed the liver capacity to clear the resulting bilirubin proportionately. The observed significant increase in total bilirubin in the formulated diet group calls for further studies in the mechanism of action of the diet.

Table 3: Effect of exposure to crude oil and treatment with *O.gratissimum* and *G. latifolium* on thyroid hormones

Groups	T_3 (ng/l)	T_4 (ug/l)	TSH(ug/ml)
Control	3.06±1.05^{b}	6.40±0.70^{a}	1.73±0.70^{a}
Formulated Diet	1.43±0.25^{a}	7.50±1.91^{a}	1.23±0.47^{a}
Crude oil	1.13±0.49^{a}	5.67±1.10^{a}	2.63±0.25^{b}
F-value	6.91	1.43	5.81
P-value	0.028	0.31	0.04

Values (mean±SD of triplicate determinations) with different superscript on same column are significantly (p<0.05) diferrent

Table 4: Effect of exposure to crude oil and treatment with *O.gratissimum* and *G. latifolium* on Lipid Profile (mMol/l)

Groups	Cholesterol	Triglyceride	VLDL	HDL	LDL
Control	1.17±.32^{a}	1.07±0.32^{a}	0.48±0.16^{b}	0.53±0.12^{a}	0.20±0.14^{a}
Formulated Diet	1.13±.31^{a}	0.63±0.25^{a}	0.19±0.067^{b}	0.47±.06^{a}	0.28±0.06^{ab}
Crude oil	1.50±.26^{a}	0.9±0.17^{a}	0.41±0.08^{ab}	0.57±0.12^{a}	0.43±0.10^{b}
F-value	1.39	2.19	5.46	0.778	3.55
P-value	0.32	0.19	0.045	0.501	0.096

Values (mean±SD of triplicate determinations) with different superscript on same column are significantly (p<0.05) diferrent

The observed changes in thyroid hormones (Table 3) were none significant in the three groups. However, the concentrations of thyroxine (T_4) and triiodothyronine (T_3) reduced none significantly in the untreated group compared to control and formulated diet groups. Decrease in these hormones could indicate inhibition in synthesis. This may have severe consequences on growth and development, since these hormones influence most metabolic and physiological processes in vertebrate organisms (Ujowundu et al., 2013). Prolonged decrease in T_3 and T_4 indicates hypothyroidism which is usually associated with elevated plasma cholesterol levels (Satyanarayana and Chakrapani, 2012).

The lipid profile parameters such as; total cholesterol, HDL-cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol fluctuated non-significantly in all the groups (Table 4). Increase in serum triglyceride in liver damage, usually is attributed to the hypoactivity of blood lipase (Ujowundu et al., 2012). This is in agreement with the non-significant fluctuations obtained in the thyroid function tests. Thyroid hormones are involved in lipid metabolism. Interference in lipid metabolism

due to liver damage and/or metabolic dysfunction affecting thyroid hormone concentration, results in ineffective lipids regulation (Cor et al., 1998; Rizos et al., 2011).

4. CONCLUSION

The activity or concentration of an antioxidant may be increased or inhibited under chemical stress depending on the intensity and duration of stress applied as well as susceptibility of exposure species (Mahmoud et al., 2011). This study has shown that exposure to crude oil, induced oxidative damage through disturbed antioxidant defense systems and increased lipid peroxidation resulting to hepatic impairment, fluctuations in thyroid and lipid metabolism. These negative influences can be ameliorated by the consumption of *O. gratissimum*and and *G. latifolium* vegeatables.

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