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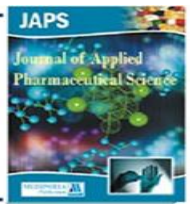

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Chemoprotective Potentials of *Ocimum gratissimum* in Diesel Petroleum induced Hepatotoxicity in Albino Wistar Rats

Ujowundu C.O., Nwokedinobi N., Kalu F.N., Nwaoguikpe R.N. and Okechukwu R.I.

Ujowundu C.O., Nwokedinobi N., Nwaoguikpe R.N.
Department of Biochemistry
Federal University of Technology
Owerri, Nigeria.

Kalu F.N.,
Department of Biochemistry,
University of Nigeria,
Nsukka, Nigeria

Okechukwu R.I.
Department of Biotechnology,
Federal University of Technology
Owerri, Nigeria.

For Correspondence
Ujowundu C.O.
Department of Biochemistry
Federal University of Technology
Owerri, Nigeria.

ABSTRACT

This study evaluated the effect of diesel petroleum intoxication in rats and the ability of phytochemicals and antioxidant content of *Ocimum gratissimum* to ameliorate such toxicity. Toxicity in rat was induced by administering 4 ml/kg body weight of diesel in the test rats except control. The intoxication and treatment with 20 % *O. gratissimum* supplemented diet was for 7 days. Serum liver function markers, Oxidative stress markers and lipid profile were estimated. Diesel induced hepatotoxicity was characterized by significant ($p < 0.05$) decrease in serum protein concentration and oxidative enzymes activities. Also increase in activities of liver function enzymes and Cholesterol was observed. The group of rats which diet was supplemented with *O. gratissimum* showed significant ($p< 0.05$) improvement in the concentration of serum proteins and decrease in the activities of liver function enzymes. Similarly the activities of oxidative enzymes significantly ($p<0.05$) increased, compared to the untreated rats. These results indicate a chemoprotective ability of this Nigerian indigenous spice.

Keywords: Hepatotoxicity, diesel, liver enzymes, oxidative stress, antioxidants.

INTRODUCTION

Many environmental contaminants are hazardous to populations of animal and plant species. There is no doubt that excessive levels of pollution cause a lot of damage to human and animal health, plants and trees as well as the wider environment. The effects on living organisms may range from mild discomfort to serious diseases such as cancer to physical deformities e.g. extra or missing limbs in frogs. Pollution effects are in most cases underestimated, thereby requiring more research to understand its effects on all life forms. Industrial pollutants have been implicated for population declines and thinning of several species (Rattner *et al.*, 1984). These pollutants and their metabolic products (no matter how small) can be carcinogenic and/or mutagenic and are potent immuno-toxicants (Boonchan *et al.*, 2000). In Nigeria, crude oil exploration pollutes the environment, thereby presenting potential hazard to both aquatic and terrestrial species (Shore and Douben, 1994). These pollutants include spilled crude oil and their refined products, effluents with traces of heavy metals, particulates and toxicants from gas flaring and green house gases. These pollutants are considered recalcitrant to (natural) biodegradation and persist in the ecosystem due to their hydrophobicity and low volatility. The effects of this pollution are varied and may include water contamination, food poisoning as such accumulating toxic chemicals from the environment to species. The ingestion of crude oil or its products orally or through polluted marine species represents a pathway for delivery of potential toxicants to the

human system. Economic activities in the areas where there are such pollutants may also be negatively affected due to inadequate water and oil effects limiting land for fishing or farming (Akpofure *et al.*, 2000). Therefore, petroleum hydrocarbon in its crude, refined or spent form has a negative impact on both human, animal and plant species (Gbadebo et al., 2009).

Ocimum gratissimum Linn (commonly called Nchanwu by igbo speaking Nigerians) is a culinary herb, indigenous to Nigeria, with a pungent sweet smell and of the family Lamiaceae (Calixto, 2000). In Nigeria the foliage is commonly used fresh in cooking recipes or added during cooking. Phytochemical studies show that both aqueous and methanolic extracts are rich in tannins, steroids, terpenoids, flavoniods and cardiac glycosides and equally has a good antoxidant activity (Afolabi *et al.*, 2007). The medicinal values of the plant lie in the component phytochemicals, which produce definite physiological actions. Phytochemicals have potential disease inhibiting capabilities and are effective in combating or preventing diseases due to their antioxidant effects (Halliwell and Gutteridge, 1992; Farombi *et al.*, 1998). Antioxidants protect other molecules (*in vivo*) from oxidation when they are exposed to free radicals and reactive oxygen species, and these have been implicated in the etiology of many diseases (Halliwell and Gutteridge, 1992; Farombi, 2000; Kasaikina, 1997; Koleva *et al.*, 2000). The impact of petroleum products which may result from exposure to different species need to be assessed on a regular basis in order to predict its possible effects on physiological characteristics of both human, animals or plants. This study seeks to determine the biochemical impact of diesel petroleum intoxication and the antioxidative and hepatoprotective effect of *Ocimum gratissimum.*

MATERIAL AND METHODS

The experimental animals used in this study were male Wistar albino rats, ages between 7 – 9 weeks old with average weight of 176.71±20.07 g. The rats were obtained from small animal holding unit of the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. The diesel used was obtained from a Nigerian National Petroleum Corporation (NNPC), fuel station in Owerri, Imo State Nigeria. The rat feed (Vital Poultry Growers Pellets; a product of Grand feed Nigeria Ltd.), the indigenous spice- *Ocimum gratissimum* leaves were purchased from Ekeonuwa Market, within Owerri municipal council, Imo State, Nigeria. All reagents used for the assays were commercial kits and products of Randox Laboratories Ltd, Antrim, United Kingdom, Biosystems S.A. Barcelona, Spain, and TECO Diagnostics, Anaheim, USA.

Diet Formulation

The fresh leaves of *O. gratissimum* were air dried in the laboratory and ground into powder and sieved through a micro pore sieve. Two hundred grams (200 g) of the powdered form *O. gratissimum* was mixed with eight hundred grams (800 g) of mashed rat feed.

Experimental Design

Eighteen (18) male Wistar albino rats were divided into three groups with each group containing six rats. The rats were housed in steel cages and after acclimatization, toxicity was induced by administering (except control) 4 ml/kg body weight of diesel by gavage. The diesel was administered to the animals every other day for 7 days. The animals were fed with the supplemented diet as they received the petroleum fraction (except control). Animals in Group I served as the control, and were not given the petroleum fraction but fed the control diet. Group II served as the test group and were given the petroleum fraction and were fed with the supplemented diet (20 % *Ocimum gratissimum* feed). Group III served as the untreated control, they were given the petroleum fraction and were fed with the normal rat feed. The Procedure for this study was in accordance with the guidelines on the care and well being of research animals (NIH, 1985) and was approved by the Department of Biochemistry Ethics Committee.

Blood Collection and Preparation of Liver Homogenate

The animals were sacrificed at the seventh day after 24 hours fast. Blood was collected by cardiac puncture and the liver was removed and refrigerated. Blood was collected with a hypodermic needle with 5 ml syringe and the blood transferred to an anticoagulant free bottle. The blood sample was kept at room temperature for 30 minutes to clot. Afterwards, the clotted blood was centrifuged to separate the serum. The liver tissues of the rats were excised, weighed and some part of it homogenized in Potassium Chloride (KCl) (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 some oxidative stress enzymes and compounds.

Biochemical Analysis

Estimation of Liver function markers

Serum albumin (ALB) was determined by the method of Doumas *et al.*, (1971), Serum total protein (TP) by the method of Tietz, (1995), Serum bilirubin was measured by colourimetric method based on the method described by Jendrassik and Grof, (1938), for the *in-vitro* determinations in serum using Randox laboratory test kit (Antrim, UK). Globulin was calculated thus; Serum Globulin = Total protein – Serum albumin (TP-ALB). The estimation of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activity was done using Reitman and Frannkel, (1957) method for the quantitative *in-vitro* determinations in serum using Randox laboratory test Kit (Antrim, UK).

Estimation of Serum Lipid Profile

The estimation of serum cholesterol was done by the combined methods of Allain et al, (1974) and Meiattini *et al.*, (1978); Triglycerides by the combined methods of Bucolo and David (1973) and Fossati and principle (1982), HDL by the method of Lopos-virella et al, (1977), for the quantitative *in-vitro* determination of lipid concentration in serum using Biosystems test kit (Barcelona, Spain). Serum LDL-cholesterol and VLDL-

cholesterol were estimation thus: VLDL (mmol/l) = Triglyceride /2.2; LDL (mmol/l) = Total cholesterol – Triglycerides/2.2 - HDL

Estimation of Oxidative Stress Makers

Catalase activity was done according to the method of Aebi (1983), Superoxide dismutase by the method of Xin *et al*, (1991); Glutathione by the method of King and Wootton (1959); Glutathione peroxidase (GPX) activity by the method of Paglia and Valentine (1967); Lipid peroxidation was estimated spectrophotometrically by measuring the concentration of the lipid peroxidation product – malondialdehyde (MDA) as described by Wall *et al*, *(*1993).

Data analysis

Results are expressed as mean ± standard deviation and all data were subjected to Analysis of Variance (ANOVA) as described by Steel and Torrie, (1960). Significant differences between the treatment means were detected at 5% confidence level using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The effects of diesel petroleum hydrocarbon and *O. gratissimum* supplemented diet on liver function marker are shown in figure 1.

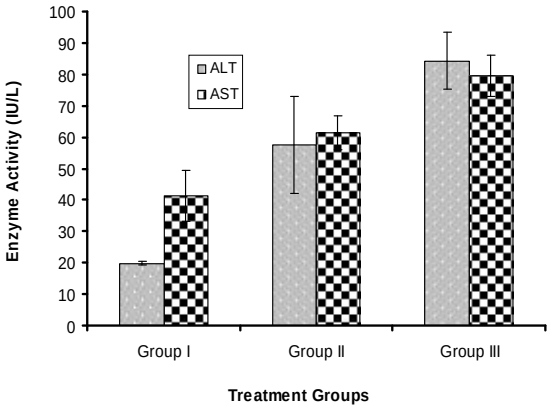

Figure 1: Effect of Diesel Petroleum and *O. gratissimum* supplemented diet on Liver function enzymes

Compared to the control (group I), the values show that the concentration of serum ALT and AST were significantly increased ($p< 0.05$) in the untreated rats (group III). The values also showed that the rats intoxicated with the diesel and treated with *O. gratissimum* supplemented diet (group III) showed significant decrease ($p < 0.05$) in serum concentration of ALT and AST. Figure 2 shows that the concentration of serum albumin, total protein, and globulin significantly decreased ($p < 0.05$) in the untreated animals when compared to control. Whereas a significant increase ($p < 0.05$) in these serum proteins was observed in the treated group. There was also a significant increase ($p < 0.05$) in concentration of serum total bilirubin (fig. 3) in the untreated animals when compared to control and treated group.

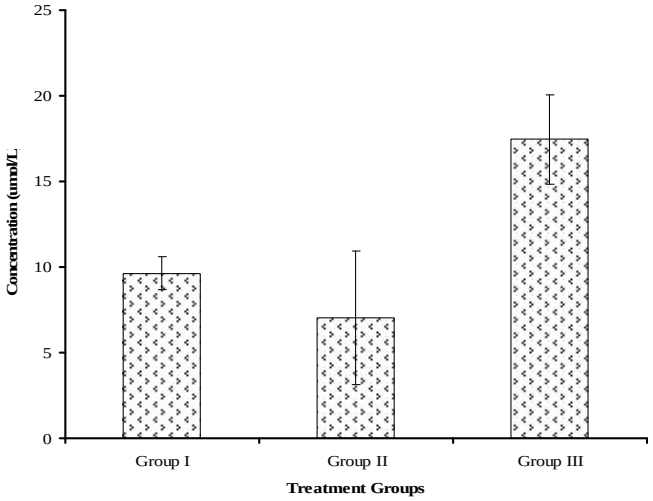

Figure 2: Effect of Diesel Petroleum Hydrocarbon intoxication and *O. gratissimum* supplemnted diet on serum Total Bilirubin

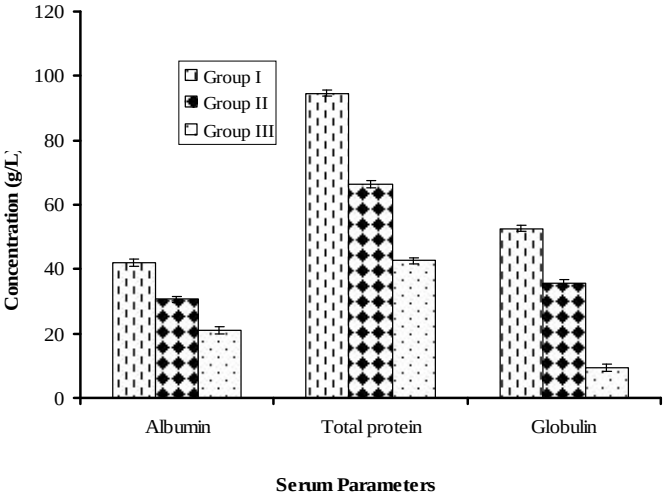

Figure 3: Effect of Diesel petroleum intoxication and *O. gratissimum* supplemented diet on serum proteins

Figure 4 shows the effects of diesel petroleum hydrocarbon and *O. gratissimum* supplemented diet on serum lipid profile. When compared to the control, serum total cholesterol concentration was elevated as a result of diesel intoxication, though not significantly ($p> 0.05$), while LDL – cholesterol concentrations increased significantly ($p= 0.04$) in the untreated rats. But rats fed with the *O. gratissimum* supplemented diet showed decrease in the values of cholesterol and LDL-cholesterol. Serum triglyceride, HDL–cholesterol and VLDL–Cholesterol concentrations showed a non significant decrease ($p> 0.05$) in the untreated rats when compared to control and treated rats. Also the treated rats showed an increase in the serum triglyceride HDL–cholesterol and VLDL – cholesterol concentrations when compared to untreated groups though this increase was not significant ($p> 0.05$). Table 1 shows the effects of diesel petroleum hydrocarbon and *O. gratissimum* supplemented diet on some oxidative stress parameters. The values shows a non significant ($p>0.0.05$) decrease in catalase activity in the untreated rats.

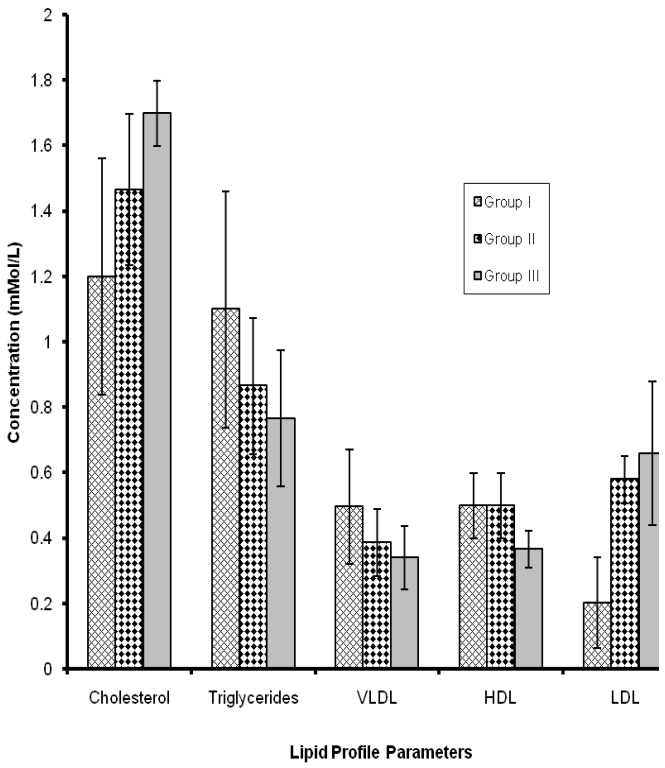

Figure 4: Effect of Diesel Petroleum hydrocarbon intoxication and *O. gratissimum* Supplementd diet on Serum Lipid Profile

Table 1: Effects of Diesel Petroleum Hydrocarbon intoxication and *O. Gratissimum* Supplemented Diet on Some Oxidative Stress Parameters.

Parameters	Group I	Group II	Group III	F-value	P-value
Catalase (IU/L)	7.81±0.37[a]	8.01±0.30[a]	7.41±0.80[a]	1.092	0.440
SOD (IU/L)	1.11±0.34[a]	1.37±0.36[ac]	0.46±0.13[b]	7.311	0.024
Glutathione (mg/dl)	2.03±0.09[a]	1.32±0.09[b]	1.43±0.28[c]	14.158	0.005
GPX (IU/L)	531.09±21.44[a]	461.57±41.21[a]	320.19±20.57[b]	40.284	0.0003
MDA (%TBARS)	5.10±1.05[a]	5.10±0.60[a]	9.65±2.50[b]	8.063	0.019

*Values with different superscripts per row are significantly different ($p<0.05$).

Whereas the treated group showed a non significant ($p>0.05$) increase in catalase activity. The activities of superoxide dismutase decreased significantly ($p=0.02$) in the untreated animals when compared to control. Also the treated rats showed increased activity of SOD when compared to the untreated group. The result shows a significant decrease ($p< 0.05$) in the concentration of glutathione and the activity of glutathione peroxidase in untreated animals when compared to control. Whereas the treated group shows a significant increase ($p< 0.05$) in the concentration of glutathione and the activity of glutathione peroxidase when compared with untreated group. The concentration of lipid peroxidation product malondialdehyde (MDA) increased significantly ($p=0.019$) in the untreated rats when compared with the control and treated rats. The concentration of MDA in the treated rats showed significant decrease ($p=0.037$) when compared with the untreated.

DISCUSSION

Diesel is one of the distilled fractions of crude petroleum which contains aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons (Henderson *et al.*, 1993). Exposure of humans and animals to crude petroleum is now on the increase because of different methods of usage, domestically and industrially. Most petroleum hydrocarbons entering the environment come from spills or leaking storage tanks.

The toxicological effects of diesel petroleum hydrocarbon may be explained as an interference with the cellular and sub cellular processes, which leads to a disruption of the normal metabolism of a living organism upon exposure to it, competing with some endogenous metabolites or inhibiting some pathways (Dede and Kagbo, 2001). Metabolism of aliphatic and aromatic hydrocarbons which constitute petroleum and petro-chemical products, like xenobiotics are mostly done in the hepatocytes (Schaumburg and Spencer, 1978). The liver is necessary for survival (Cory *et al.*, 1998). It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis and detoxifications (Ihedioha and Chineme, 2005).

The elevated serum ALT and AST concentration in untreated rats when compared to control could be attributed to damage of the structural integrity of liver (Rosen and Keefe, 2000; Friedman *et al.*, 2003; Chenoweth and Hake, 1962). It also indicates that the diesel could have induced necrotic lesions in the hepatocytes of the rats. Similarly, the decrease in serum albumin, total protein and globulin and the increase in total bilirubin in the untreated animals further suggest a possible liver cell damage or bile duct damage. Thus, indicating that the synthetic function of the liver may have been markedly diminished by diesel intoxication (Green and Flamm, 2002). The decrease in serum ALT and AST concentrations in the treated animals may be due to the protective influence of *O. gratissimum* supplemented diet on liver cells following restoration of liver cell membrane permeability (Kalab and Krechler, 1997). This protective effect is indicated by the reduction in the activities of the enzymes in the extracellular milieu of the liver cells. This is in agreement with the findings of Kataria and Singh, 1997; Mathur, 1994) who reported that herbs have hepatoprotective effects on chemically induced hepatic damage in rats. The rats treated with the *O.gratissimum* supplemented diet showed some kind of ameliorative effect by the elevatation of the serum albumin, total protein and globulin concentrations and also decreasing the concentration of total bilirubin. This is in agreement with the findings of Faiyaz and Asna, (2010) whodiscovered that *Freeus racemosa* possesses potent hepatoprotective effects against CCl_4 – induced hepatic damage in rats. The changes in the hepatic cells caused by the diesel intoxication may have resulted in the generation of free radicals species from the metabolism of aliphatic and aromatic hydrocarbon content of the diesel (Leighton *et al.*, 1985; Bondy *et al.*, 1995). Oxidative stress occurs when there is an imbalance between production and scavenging of free radicals.

Antioxidant enzyme dependent defenses play important role in scavenging free radicals produced under oxidative stress.

The values of the reduced activities of catalase, superoxide dismutase and glutathione peroxidase in the rats intoxicated with diesel and not treated indicates oxidative stress (Eriyamremu *et al.*, 2007). Similarly the decrease in the concentration of glutathione and increase in the concentration of lipid peroxidation product (MDA) further suggest oxidative stress of the status in the rats. Treatment of the rats with *O. gratissimum* supplemented diet, significantly reduced lipid peroxidation and increased the activities of the antioxidative enzymes indicating modification of oxidative stress. The protection of liver cells against toxic materials that generates free radicals may decrease inflammation (Yang *et al.*, 2000). This elevation in the activities of antioxidative enzymes in the treated rats when compared to the untreated rats could be due to actions of antioxidative compounds present in *O. gratissimum* as noted by Afolabi *et al*., (2007). This also agrees with the findings of Sumitra et al, (2001) who reported the antioxidative, antilipoperoxidative and increase in antioxidant enzyme content of the liver observed with Arjunolic acid and flavonoids present in arjuna.

The elevation of serum cholesterol in the untreated rats may be the result of significant concentration of serum LDL – cholesterol (Grundy, 1987) and this is a risk factor for coronary heart disease (CHD) (NIHDC, 1985). The increased concentration in some of the serum lipids may also be attributed to increased lipolysis, mediated by increased Norepinephrine release which act through interference with the intracellular functions of Ca^{2+} in the cytoplasm (Anad, 2005). All these metabolic activities may lead to increased synthesis of reactive oxygen species (ROS), inducing oxidative stress resulting in metabolic dysfunction (Auer, 1990).The reduced concentration of cholesterol and LDL-cholesterol in the rats fed with the *O. gratissimum* supplemented diet indicates that *O. gratissimum* may have increased uptake and metabolism. LDL particles and cholesterol are absorbed from the bloodstream by receptors on the cells, internalized (by receptors on liver cells) and broken down. For this process to occur, the liver integrity most have been restored. The increased HDL–cholesterol in the treated rats further supports the restoration of the liver integrity Lipid metabolism is affected once there is liver damage since the disturbance of cell membrane integrity is likely to cause some membrane lipids to be released into circulation and this causes tissues to compromise their effectiveness in regulating lipid metabolism. There is therefore, the likelihood that exposure to diesel predisposes the subject to atherosclerotic conditions. Similarly, the insignificant decrease in serum triglyceride could be as a result of several clinical conditions like chronic liver damage which can be responsible for mal absorption. Thus, the observed hypercholesterolemia could be due to liver damage, since there were alterations in the activities of ALT, AST and plasma proteins concentrations in experimental animals following diesel petroleum exposure.

Serum triglyceride and VLDL–Cholesterol concentrations showed a non significant decrease ($p< 0.05$) in the untreated rats when compared with control and treated rats. This is contrary to other reports which indicated increased serum triglyceride as a result of liver toxicity (Amrita *et al.*, 2008; Metwally, 2009). Increase in serum triglyceride in liver damage is attributed to hypoactivity of lipoprotein lipase in blood vessels which metabolises triglycerides. High serum triglyceride concentration along with decreased absorption of fatty acids by the adipose tissue is associated with low level of HDL, insulin resistance and increased risk of atherosclerosis **(**Terasawa *et al.*, 2000).

This study has shown that diesel petroleum hydrocarbon intoxication could result to hepatocellular damage and the potential to elicit the production of ROS. It can also be stated the phytochemical and antioxidant constituents of *O. gratissimum* can influence and restore cellular functions as well as structural integrity of the liver.

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