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The Antioxidative Potentials of *Gongronema latifolium* on Diesel Petroleum Induced Hepatotoxicity

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

Ujowundu C.O., 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.

Ihejirika C.E.
Department of Environmental Technology Federal University of Technology Owerri, NIGERIA.

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

ABSTRACT

In Nigeria, petroleum contamination of the environment occurs through accidental spillage and/or sabotage of petroleum pipelines. Human exposures to petroleum products occur frequently from occupational and domestic usage. In this study male albino rats were used to evaluate the antioxidative effects of *Gongronema latifolium* supplemented diet on diesel petroleum induced toxicity resulting from exposure. The activities of alanine aminotransferase, aspartate aminotransferase and concentrations of total bilirubin and malondialdehyde increased ($p<0.05$) in the animals exposed to the petroleum product. The activities of some oxidative stress enzymes and concentrations of serum proteins decreased ($p<0.05$) in exposed animals. The activities of liver function enzymes and oxidative stress parameters obtained in the animals which received *G. latifolium* supplemented diet with diesel intoxication were comparable to the control, indicating a protective role of *G. latifolium*.

Key words: Diesel petroleum, oil spill, hepatotoxicity**,** liver function, oxidative stress

INTRODUCTION

Petroleum hydrocarbon in its crude, refined or spent form has negative impact on both, human, animal and plant species (Clark, 1992). The presence of crude and refined oils in the environment through spill most of the time is accidental while its deliberate introduction into the environment is often termed sabotage. The toxicity of hydrocarbons is directly related to their physical properties, specifically the viscosity, volatility, surface tension, and chemical activity of the side chains. Organ & systems that can be affected by hydrocarbons include the pulmonary, neurologic, cardiac, gastrointestinal, hepatic, renal, dermatologic, and hematologic systems (Akubue, 2007). Diesel is very toxic and highly flammable, the main routes of exposure of humans and animals to hydrocarbon toxicity includes inhalation, ingestion, and absorption. Hepatotoxic effects can be minimized or prevented or eliminated by certain active compounds serving as valuable antioxidants obtainable from natural plant resources (Akhatar, and Ali, 1984). *G. latifolium*, a perennial climber crop, native of the humid tropic of south eastern Nigeria (Okafor, 1989) known locally among the Efik, Ibibio and Igbo speaking communities of Nigeria as 'Utazi' could have some augumentary or protective effect against certain hepatocellular injury and also the leaves posses antioxidant activity (Ugochukwu, and Babady, 2003).

MATERIAL AND METHODS

The animals used are male Wistar albino rats of 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.). *G. latifolium* leaves a Nigerian indigenous spice was purchased from Ekeonuwa Market, in 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.

Preparation of Diet and Formulation of 20 % *G. latifolium*

Fresh leaves of *G. latifolium* 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 of *G. latifolium* was mixed with eight hundred grams (800 g) of mashed rat feed.

Experimental Design

Acute toxicity test of diesel was carried out using Wistar albino rats. Rats were grouped into five with three rats per group and were treated orally with 1, 2, 4, 6, and 9 ml/kg body weight of diesel respectively. The rats were observed over a 24 hour period for nervousness, dullness, weight loss, in-coordination and or death. Increased dullness and weight loss was observed with increased diesel intake and death occurred at 9 ml/kg. From the range of doses used, 4 ml/kg body weight was chosen for this study. Eighteen (18) male Wistar albino rats divided into three groups with each group containing six rats. The rats were housed in steel cages and allowed to acclimatize. Oxidative stress was induced by administering (except control) 4 ml/kg body weight of diesel orally, using a 3.5 cm feeding tube attached to a syringe. Diesel was administered to the animals every other day for 7days. 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 was fed with the supplemented diet (20 % *G. latifolium* feed). Group III served as the untreated control, they were given the petroleum fraction and were fed with the normal grounded rat feed. The Procedure for this study was in accordance with the guidelines on the care and well being of research animals and was approved by the Department of Biochemistry Ethics Committee.

Preparation of Serum and Liver Homogenate

The rats were sacrificed on the eight 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 portion 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 for some oxidative stress enzymes and compounds.

Estimation of Serum Liver Function markers

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

Estimation of Serum Lipid Profile

Serum cholesterol was estimated 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 Lopes-virella et al, (1977). These estimations were done according to 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 estimated 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 was estimated by the method of Paglia and Valentine, (1967); Lipid peroxidation was estimated spectrophotometrically (Wallin et al, 1993).

Data analysis

Results are expressed as mean ± standard deviation and all data were subjected to Analysis of Variance (Steel and Torrie, 1960) and significant differences between the treatment means were detected at ($p \leq 0.05$).

RESULTS

Effect of Diesel intoxication and *G. latifolium* on the Activities of Serum Liver Function Enzymes and Concentration of Proteins and Bilirubin

Figure 1a shows that the activities of ALT (84.33±11.24 IU/L) and AST (79.66±8.08 IU/L) in diesel intoxicated but untreated animals increased significantly ($p<0.05$) when compared to the activities of ALT (19.86±0.57 IU/L) and AST (41.33±9.81 IU/L) in the control and the ALT (35.23±1.98 IU/L) and AST

(66.33±3.05 IU/L) in the treated group. Similarly the concentration of total protein, globulin and albumin (Figure 1b) in the untreated group decreased significantly ($p<0.05$). Also the concentration of total bilirubin (Figure 1c) in diesel intoxicated but untreated animals increased significantly ($p<0.05$) when compared to the control and treated animals. These results showed that the animals treated with *G. latifolium* showed significant increase ($p < 0.05$) in the concentrations of serum proteins and decrease in total bilirubin compared with the untreated animals. Significant decrease ($p<0.05$) was also observed in the activities of serum ALT and AST in the treated animals compared with the untreated.

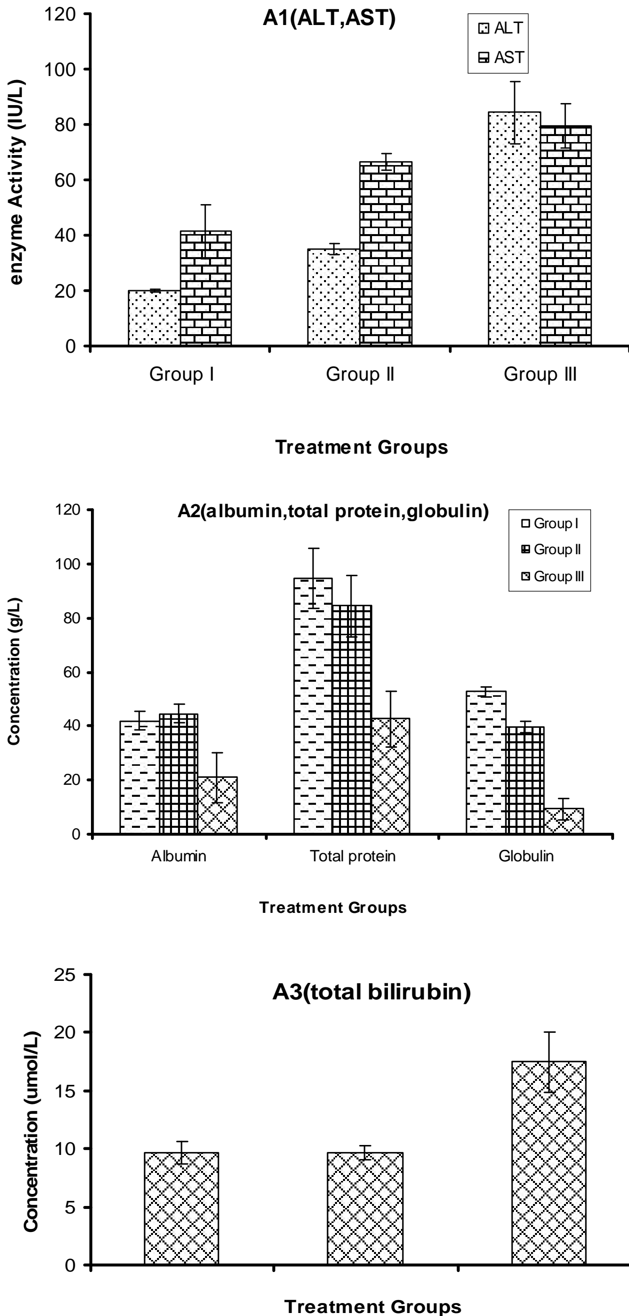
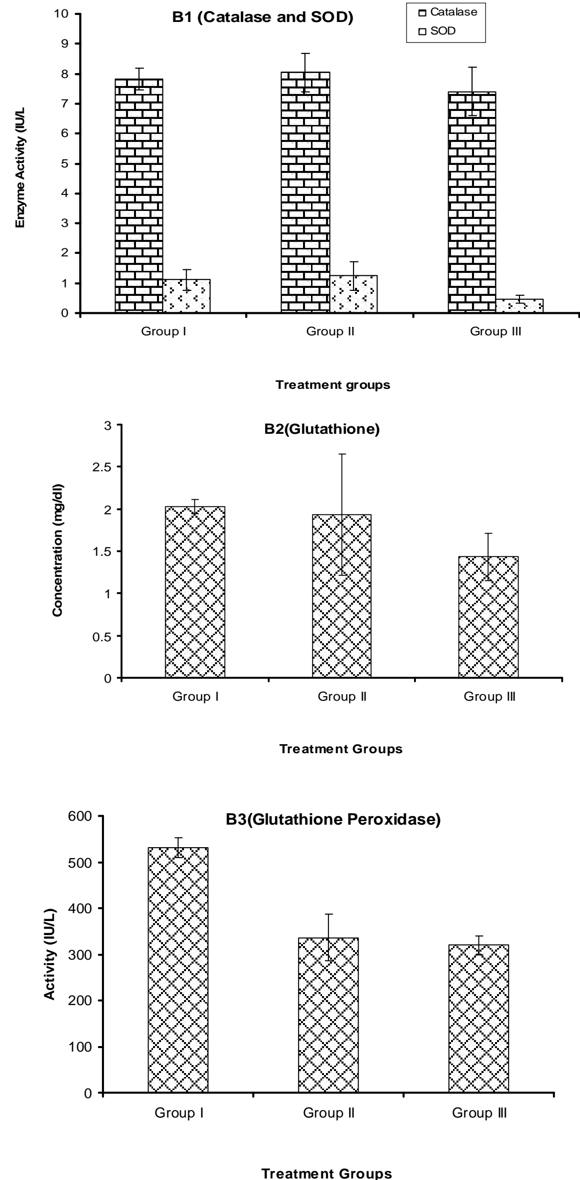


Fig 1 (A1-A3) Effect of Diesel intoxication and *G. latifolium* on serum (A1) AST and ALT, (A2) albumin, total protein and (A3) globulin.

Effect of Diesel intoxication and *G. Latifolium* on oxidative stress parameters

The values of the results obtained shows that the activities of catalase, superoxide dismutase (Figure 2a) and glutathione peroxidase (Figure 2b) in the untreated animals decreased ($p>0.05$) when compared with control and the treated groups. The result (Figure 2c) of glutathione concentration (1.932±0.72 mg/l) in the animals administered diesel petroleum and treated simultaneously with *G. latifolium* is maintained within concentration of the animals in the control (2.03±0.09 mg/l) group. Figure 2d shows that malondialdehyde concentration in the untreated group increased significantly (9.65±2.50 %TBARS) when compared to control (5.10±1.05 %TBARS) and the treated group (4.60±0.53 %TBARS). The treated groups showed elevated activities of antioxidative enzymes and elevated concentration of glutathione when compared with the untreated group.



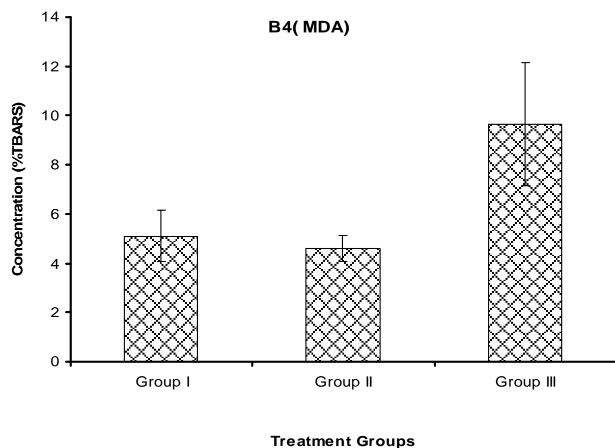


Fig 2 (B1-B4) Effect of Diesel intoxication and *G. Latifolium* on oxidative stress enzyme activity and lipid peroxidation.

Effect of Diesel intoxication and *G. Latifolium* on serum lipid profile

Figure 3 shows that LDL-cholesterol and total cholesterol concentrations in the untreated group increased (p<0.0.05) when compared to control. HDL-cholesterol, VLDL-cholesterol and triglyceride concentrationa decreased significantly ($p<0.05$) when compared to control and the treated group.

But rats treated with the supplemented diet showed decrease in the values of cholesterol and LDL-cholesterol.

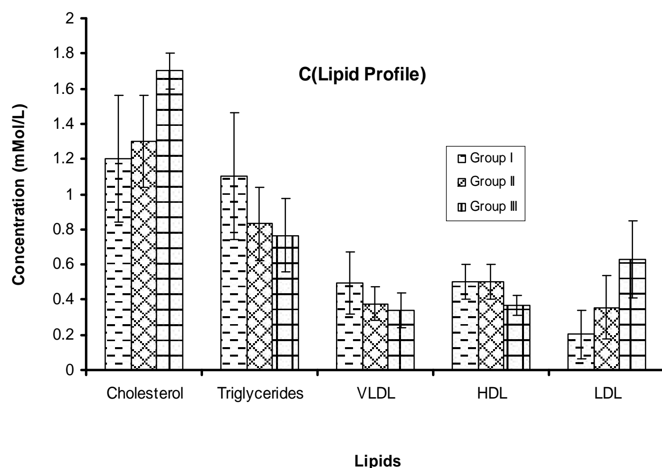


Fig 3 (C) Effect of Diesel intoxication and *G. Latifolium* on serum lipid profile

DISCUSSION

The liver has a central role in the maintenance of lipid homeostasis and the presence of toxicants may alter the concentration of serum lipids which could increase the risk of atherosclerosis, given that increased LDL cholesterol and decreased HDL cholesterol are implicating risk factors of atherosclerosis and related cardiovascular diseases (Crouse and Grurdy, 1984).

The elevated activities of Alanine aminotransferase, aspartate aminotransferase and increased concentration of total bilirubin in the diesel intoxicated rats indicates necrosis and compromised integrity of liver cell membranes (Mukherjee, 2003). The reduced ($p<0.05$) activities of ALT, AST and concentration of total bilirubin in rats which diet was supplemented with *G. latifolium* (treated) compared to the untreated could be attributed to the ability of *G. latifolium* to prevent hepatic damage. It may have also initiated the healing and regeneration of liver parenchyma and cells, respectively (Thabrew *et al.*, 1987). The decrease in the total protein, globulin and albumin concentration in the untreated animals and the improvement in the treated animals, confirms the hepatotoxicity of diesel and hepatoprotective ability of *G. latifolium*. The decrease in serum protein concentration can be attributed to loss of synthetic ability by the hepatocytes (Deepak *et al.*, 2000). *G. latifolium* phytochemical and antioxidant contents may have decreased the metabolism of diesel into more toxic metabolite. This could reduce the production of free radicals and boast the activities of free radical scavengers (Chung *et al.*, 1999), minimizing hepatocellular injury.

The production of free radicals by diesel intoxication was indicated in this study from the observed significant decrease ($P < 0.05$) in activities of catalase, superoxide dismutase and glutathione peroxidase and concentration of glutathione in the untreated group. One important effect of free radicals activity is lipid peroxidation (Morel *et al.*, 1983), which produces malondialdehyde. The result shows that diesel significantly increase the concentration of MDA in the untreated group. Oxidative stress causes lipid peroxidation and membrane damage. This leads to oxidation of glutathione and consequently ATP and NADPH depletion that culminate to disruption in lipid synthesis and transport. Free radicals could cause oxidative stress which may damage cellular compounds such as DNA, carbohydrate, protein and lipid (Buttner and Bums, 1996). Antioxidant enzymes, such as the glutathione peroxidase family, protect cell surfaces, extracellular fluid components and other enzymes from oxidative stress by catalyzing the reduction of hydrogen peroxide, lipid peroxide and organic hydroperoxide using reduced glutathione (Schafer, 2001). The elevated activities of glutathione peroxidase in the treated group compared with the untreated suggest a protective capacity of the antioxidant systems. The elevated concentration of glutathione and the activities of catalase, superoxide dismutase, glutathione peroxidase in the treated animals indicate a mutually supportive team of defense against reactive oxygen species (Nirmala *et al.*, 2011). This defense is initiated by the bioactive phytochemicals and other antioxidant compounds in *G. latifolium* (Morebise *et al.*, 2002). This is in agreement with study on *Saacia oblonga* wall extract, which showed the importance of phytochemicals in the amelioration of oxidative stress (Krishnakumar *et al.*, 1998).

The significant increase in the concentrations of serum total cholesterol and low density lipoprotein-cholesterol in the untreated animals is an indication of disturbance in lipid metabolism. Membrane peroxidation may have altered the activities of liver enzymes involved in cholesterol metabolism and lipoprotein formation resulting in higher total serum cholesterol.

HDL-Cholesterol, very low density lipoprotein and triglyceride ocncentration decreased in untreated rats when compared to the treated rats. This unconventional fluctuation in serum lipid may be the effect of liver damage, which causes tissue to compromise its effectiveness in regulating lipid metabolism (Cory *et al.*, 1998). There is therefore a likelihood that exposure to the diesel predisposed the rats to atherosclerotic conditions. The attainment of near normalcy in the concentration of HDL-cholesterol in the treated animals signifies hypolipidemic potential of *G. latifolium.*

CONCLUSION

The results obtained from this study suggest diesel petroleum induced hepatic damage probably by the production of reactive oxygen species which was elevated significantly. However, *G. latifolium* conferred protection on the liver by the antioxidative action of the bioactive constituents of *G. latifolium* which maintained the antioxidant enzyme activities close to normal.

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