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Management of Changes in Liver Chemistry in Male Rats Acutely Exposed to Crude Petroleum Oil

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ABSTRACT

This Study investigated the protective effect of *Gongronema latifolium* supplemented diet against acute hepatotoxicity induced by crude petroleum oil on male rats. Oral exposure of rats to crude petroleum significantly decreased ($P<0.05$) the activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD). Significant decrease in concentrations of glutathione and increase in malondialdehyde (MDA) was observed also, but catalase activity decreased non-significantly. Activities of these oxidative stress enzymes and concentrations of glutathione and MDA in rats exposed to crude petroleum and fed simultaneously with 20% *G. latifolium* supplemented diet were close to those obtained in control rats. Fluctuations in liver function parameters and serum lipid profile were observed. These results indicate that consumption of *G. latifolium* supplemented diet has protective effect against crude oil induced liver tissue damage.

Keywords: Hepatotoxicity, oxidative enzymes, crude oil, liver function enzymes, *Gongronema latifolium.*

INTRODUCTION

Crude oil contains significantly high amount of toxic chemicals which can cause a wide range of health effects in people and wildlife, depending on the level of exposure and susceptibility. The chemicals can impair normal growth and development through a variety of mechanisms. They can cause mutations that may lead to cancer and multi-generational birth defects. The polycyclic aromatic hydrocarbon content of crude consists of fused aromatic rings (Fetzer, 2000). Some are identified as carcinogens, mutagens, and teratogens, for example benzene (CDC, 1999). Exposure of humans and animals to these chemicals is increasing in terms of the environmental level and the different usage of crude oil (Patrick-Iwuanyanwu *et al.,* 2011). Difference in exposure or contact will occur based on location, work, personal activities, age, diet, use of protective equipment and other factors. The toxic effect can be acute lethal, sub-lethal or both, depending on the level of exposure, organism exposed and the dosage it is exposed to (Rothman *et al.,* 1996).

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Gongronema latifolium is a commonly consumed spice in Nigeria with useful medicinal potential. It has high level of phytochemicals which confers it antibacterial, antitumour, anticancer and antidiabetic properties (Okafor *et al.,* 1993; Zhejian *et al.,* 2007). In Nigeria, especially in Niger Delta region this spice is used in the preparation of soups and stews and in herbal concoctions. Research studies have been carried out to determine the effects of crude oil on rats and catfish (Sunmonu & Oloyede, 2006; 2007). But the authors have failed to utilize the plant resources abundant in our environment to ameliorate the toxicological impact of crude oil and its fractions.

This study was designed to investigate the biochemical and toxicological effect of readily available plant and plant product *G. latifolium* supplemented diet in the presence of crude oil induced intoxication.

MATERIALS AND METHODS

Plant materials
Collection, identification and preparation of plant materials
Gongronema latifolium leaves were purchased from Ekeonuwa Market in Owerri municipal council, Imo State, Nigeria. The plant was identified by Dr. A.C. Ibe, a plant taxonomist in the Department of Crop Science, Federal University of Technology Owerri, Nigeria. The fresh leaves were air dried in the laboratory, ground into powder and sieved. Powdered *G. latifolium* leaves were mixed with mashed rat feed (1:4 ratios) to obtain 25 % *G. latifolium* supplemented feed. The feed mixture was made into pellets and stored.

Experimental animals and crude petroleum
Healthy male albino rats (*Rattus norvegicus*) of 7–9 weeks old, with an average weight of 176.71±20.07 g were obtained from the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. The rats were kept in well ventilated steel cages under natural environmental conditions of temperature and 12 hours light/dark cycle. Rat feed and water was provided *ad libitium*. This study followed the guidelines on the care and well being of research animals (NIH, 1985) and was approved by the Ethical Committee of the Department of Biochemistry Federal University of Technology Owerri, Nigeria. Crude petroleum was obtained from a Nigerian National Petroleum Corporation (NNPC), refinery, Portharcourt, Rivers State, Nigeria. Rat feed was from Vital Poultry Growers Pellets (a product of Grand feed Nigeria Ltd.).

Chemicals and Reagents
Chemicals used in this study were of analytical grade. All reagents 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.

Preliminary Study
Preliminary toxicity study to determine the volume of crude oil that could cause toxicity was carried out using 15 healthy albino rats. Rats were divided into five groups of three rats per group and were treated orally with 1, 2, 4, 6, and 9 ml/kg body weight of crude oil 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 was chosen for this study.

Hepatoprotective study
Eighteen male albino rats were grouped into three with each group containing six rats. They were allowed to acclimatize for two weeks. Oxidative stress was induced by administering (except control) 4 ml/kg body weight of crude oil orally, using a 3.5 cm feeding tube attached to a syringe. Crude oil was administered to the animals every other day for 7days. Animals were fed with supplemented diet as they received the crude oil (except control). Rats in group I served as control and they were fed control diet (Rat feed only) and not administered crude oil. Rats in group II served as test group and were administered crude oil and supplemented diet (25 % *G. latifolium*). Group III served as the untreated control and were administered crude oil and fed control diet.

Blood Collection and Preparation of Liver Homogenate
The animals were sacrificed after 24 hours fast on the eighth day. They were anaesthetized by dropping into a jar containing cotton wool soaked in chloroform. Blood was collected by cardiac puncture, allowed to coagulate at 37 °C for 30 min and serum separated by centrifugation at 2500 rpm for 10 min and stored at 4 °C. Liver tissues of rats were excised, weighed and some part 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 oxidative stress enzymes and compounds.

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 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 aminotransaminase (ALT) and aspartate aminotransaminase (AST) activities was 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

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), high density lipoprotein (HDL) - cholesterol by the method of Lopes-virella et al, (1977), for the quantitative *in-vitro* determination of lipid concentration in serum using Biosystems test kit (Barcelona, Spain). Serum low density lipoprotein (LDL) - cholesterol and very low density lipoprotein (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 (SOD) by the method of Xin et al, (1991); glutathione (GSH) 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 Wallin 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

Table 1 presents the effects of crude oil and *G. latifolium* supplemented diet on Oxidative stress parameters. It shows a significant (P<0.05) effect of oral ingestion of crude petroleum on the activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and concentration of glutathione and malondialdehyde. Catalase activity decreased non-significantly. The result shows that rats (in group II) which consumed *G. latifolium* in the diet with the oral administration of crude oil expressed oxidative stress properties which are within those of the control animals (group I) which were maintained on the rat feed only.

The effect of crude petroleum and *G. latifolium* is shown in table 2. Animals administered crude petroleum only (group III) showed significant (P<0.05) increase in activities of serum hepatic enzymes, ALT and AST. Concentrations of total protein and globulin decreased significantly (P<0.05) and total bilirubin increased significantly in group III rats when compared to control rats. Serum albumin concentration was non-significantly affected. The values obtained from feeding rats with supplemented diet showed non-significant (P<0.05) change in AST and ALT activities and concentrations of albumin and total bilirubin, whereas concentrations of total protein and globulin decreased significantly when compared to control rats. Thus showing that G.

latifolium maintained these serum hepatic parameters within normal.

Effect of oral administration crude oil and feeding with *G. latifolium* supplemented diet on lipid profile is shown in table 3. A general view of the values obtained showed that the lipid profile of rats were non-significantly (P<0.05) affected by any of the treatments. On group comparison only LDL–cholesterol was significantly increased in group III when compared to control (group I).

DISCUSSION

Studies have implicated free radicals from exogenous and indigenous sources in the etiology of a lot of degenerative diseases such as coronary artery diseases, stroke, rheumatoid arthritis, diabetes and cancer (Halliwell *et al.,* 1992). In this study the significantly reduced activities of SOD and GPx and concentration of glutathione and MDA may have resulted from by hepatic injury from oxidative stress from crude petroleum oil exposure. The non-significant values obtained from rats fed with *G. latifolium* and when compared to control indicates antioxidative and hepatoprotective properties *G. latifolium*. The activity of catalase was not adversely affected by either the diesel or the treatment. The near normal activity of SOD and GPx in rats treated simultaneously with *G. latifolium* shows that the spice contains active principles that inhibit actions of oxygen free radicals (Demicheli *et al.,* 2007). In system, tissue and organ damage, reduced glutathione (GSH) makes up the first line of defense against free radicals resulting from xenobiotic ingestion. The drop in the concentration of liver GSH and corresponding increase in concentration of malondialdehyde and decrease in activity of glutathione peroxidase indicates hepatocytes damage. *G. latifolium* fed rats showed significant increase in GSH activity, suggesting a possible effect on the de novo synthesis and or regeneration of GSH. Ko et al (1995) reported GSH homeostasis in the presence of increased radicals. Increased MDA indicates increased lipid peroxidation which could have resulted from depletion of GSH concentration. The maintenance of MDA concentration of treated rats close to the concentration of control rats implies that *G. latifolium* inhibited the oxidation of the thiol groups, thus preventing GSH depletion. This indicates antioxidative role of the *G. latifolium* in the supplemented diet. The consequence of the oxidative effect of diesel contamination was observed from the significant increase in activities of liver damage marker enzymes – AST and ALT. This increase in activities of these enzymes indicated cellular leakage and failure of functional integrity of liver cell membranes (Mukherjere, 2003). The reduced activities of AST and ALT in rats fed supplemented diet suggest protective effect of *G. latifolium*. The active constituents of the plants may have caused a stabilization and repair of plasma membranes damaged by exposure to contamination (Thabrew *et al.,* 1987).

The observed significant decrease in total protein and globulin concentrations in crude petroleum treated rats and the non-significant decrease in concentration of albumin in this study

Table. 1: Effect of crude oil intoxication and G. latifolium supplemented diet on Oxidant parameters.

	Catalase (IU/g tissue)	Superoxide Dismutase (IU/g tissue)	Glutathione (mg/g tissue)	Glutathione Peroxidase (IU/g tissue)	MDA (%TBARS)
Group I	7.81 ± 0.37^a	1.11 ± 0.34^a	2.03 ± 0.09^a	531.09 ± 21.44^a	5.10 ± 1.05^a
Group II	8.06 ± 0.25^a	1.11 ± 0.06^a	1.73 ± 0.23^a	427.35 ± 84.91^a	3.75 ± 0.25^a
Group III	7.56 ± 0.44^a	0.37 ± 0.33^b	1.17 ± 0.09^b	318.80 ± 16.24^b	7.08 ± 0.80^b
F value	1.47	9.484	24.39	12.78	13.97
P value	0.30	0.01	0.001	0.006	0.005

Values are mean ± Standard deviation. Values with different superscripts per column are significantly different (P<0.05).

Table. 2: Effect of crude oil intoxication and *G. latifolium* supplemented diet on Liver function parameters.

	Albumin(g/l)	Total protein (g/l)	Globulin (µmol/l)	Total Bilirubin (g/l)	ALT (IU/l)	AST (IU/l)
Group I	42.00 ± 3.61^a	94.67 ± 9.29^a	52.67 ± 5.77^a	9.63 ± 0.94^a	19.86 ± 0.57^a	41.33 ± 9.81^a
Group II	40.33 ± 1.24^a	72.00 ± 5.58^b	31.66 ± 4.92^b	14.86 ± 2.68^{ab}	21.80 ± 1.47^a	63.66 ± 11.67^a
Group III	42.33 ± 2.62^a	73.00 ± 3.56^b	30.66 ± 2.4^b	19.86 ± 0.46^b	31.93 ± 6.38^b	82.00 ± 7.78^b
F value	0.402	9.383	17.582	4.4604	5.84	9.531
P value	0.685	0.014	0.003	0.061	0.038	0.013

Values are mean ± Standard deviation. Values with different superscripts per column are significantly different (P<0.05).

Table. 3: Effect of crude oil intoxication and *G. latifolium* supplemented diet on Liver Lipid Profile.

	Cholesterol (mMol/l)	Triglycerides (mMol/l)	VLDL (mMol/l)	HDL (mMol/l)	LDL (mMol/l)
Group I	1.20 ± 0.36^a	1.10 ± 0.36^a	0.50 ± 0.10^a	0.50 ± 0.10^a	0.20 ± 0.14^a
Group II	1.26 ± 0.38^a	0.53 ± 0.32^a	0.24 ± 0.15^a	0.56 ± 0.15^a	0.35 ± 0.04^a
Group III	1.36 ± 0.49^a	0.87 ± 0.21^a	0.38 ± 0.10^a	0.60 ± 0.10^a	0.41 ± 0.12^b
F value	0.694	2.63	2.31	0.538	3.05
P value	0.535	0.150	0.180	0.609	0.121

Values are mean ± Standard deviation. Values with different superscripts per column are significantly different (P<0.05).

is in agreement with that obtained in similar studies when rats were exposed to crude oil (Sunmonu and Oloyede, 2007; Braid *et al.,* 2011). In rats intoxicated with crude oil and fed supplemented albumin concentrations were maintained close to that of control. Concentration of total bilirubin increased significantly in exposed and untreated rats indicating inhibition of binding, conjugation and excretory capacity of hepatocytes (Mankani *et al.,* 2005), because liver cells are responsible for removing bilirubin from serum (Nelson and Cox, 2005). However the non significant increase in total bilirubin concentration obtained in rats fed supplemented diet could be linked to the anti-hepatotoxic and liver protective ability of *G. latifolium*. Related reduction in bilirubin concentration on treatment with plant and plant products have been reported by Rosalinda *et al*. (2009) and Tsala *et al*. (2010). *G. latifolium* constituents may have protected the liver by enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts.

The serum lipid profile (except LDL-cholesterol) of all the experimental groups did not show significant change. Fluctuations in lipid profile are very important in monitoring incidences of cardiovascular disorders. Serum concentrations of total cholesterol, triglycerides, LDL- cholesterol and HDL-cholesterol are independent but are significant predictors of cardiovascular disease risk (Wilson, *et al.,* 1998). A study by Brunzel *et al.,* (2008) showed that increased concentration of LDL-cholesterol signified oxidative stress. The significant increase in LDL-cholesterol concentrations observed in rats exposed to crude oil but not fed with supplemented diet emphasizes the observation of Brunzel *et al.,* (2008). LDL-cholesterol concentration in rats fed *G. latifolium* supplemented diet did not cause a significant change compared to control rats. The observed values of lipids are in line with the work of Alisi et al.*,* (2008), which reported a causual association between hypolipidemic potential and hepatoprotective effect. But Nwachukwu and Ibeh (2009) have reported

hypolipidemic effect of *G. latifolium*. The non-significant effect on crude petroleum and *G. latifolium* treatment could be that the mechanism involving lipid metabolism may not have been altered or disturbed by these exposures. It is a fact that biochemical synthesis and/or degradation machinery can only cause observable changes when stimulated (Akpanabiatu *et al.,* 2005).

CONCLUSION

The values of oxidative and liver function parameters obtained in this study indicates that administration of *G. latifolium* confers protection against contents of crude oil which induces liver tissue disturbances and/or damage through the regulation of oxidative parameters by the antioxidants present in *G. latifolium*

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