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Effect of Cassava based diet on lipids concentration in albino rats fed with crude oil contaminated diet

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ABSTRACT: The study was carried out to ascertain the effect of a cassava based diet (gari) on lipid profile in albino rats fed crude oil contaminated diets by feeding diet contaminated with various concentrations of crude oil mixed with 20% gari to albino rats to determine the protective effect of gari. The lipid profile (Cholesterol, triglycerides, HDL cholesterol and LDL cholesterol) were monitored in the animals. Gari feeding at 20% caused dose dependent reduction in Cholesterol and LDL cholesterol with dose dependent increases in Triglycerides and HDL cholesterol in gari fed albino rats compared with Petroleum fed albino rats ($P<0.05$) suggesting that gari reversed the effect of crude oil on changes in lipid profile. Dose dependent increase in cholesterol, LDL cholesterol and dose dependent decrease triglycerides and HDL cholesterol was observed in petroleum fed rats compared with their controls ($P<0.05$). The study showed that ingestion of petroleum contaminated diet caused increase cholesterol and LDL cholesterol and decreased triglycerides and HDL cholesterol, but supplementation of the diet with 20%Gari lowered the increased concentrations of cholesterol and LDL cholesterol observed in the Petroleum contaminated diet while increasing the triglycerides and HDL cholesterol concentrations. This study showed that feeding on gari diet caused reversed to changes in lipid concentration caused by crude petroleum. © JASEM

Nigeria is a major petroleum producing country. One drastic effect associated with its exploration and exploitation is the contamination of the immediate environment with petroleum hydrocarbons (Amadi et al, 1993). Crude petroleum contains hundreds of compounds and the chemical composition varies between geologic formations (Coppock *et al* 1995). They have also been grouped into types as light, medium (Intermediate) and heavy depending on their density, physical and chemical properties. The route of administration is mostly oral and external application for burns and wounds. As aromatic hydrocarbons are relatively soluble in water (Volkman, et al 1994), it is expected that the potential of this light crude oil to have adverse toxic effects is higher than for heavier, less water–soluble crude oils. The ingestion of petroleum hydrocarbon has been reported to induce oxidative stress (Val and Almeida-Val, 1999) through the generation of free radical (Achuba and Osakwe, 2003). It has been established that free radical generation with subsequent oxidative modification leads to lipid peroxidation (Halliwell, 1994) that damages critical cellular macromdecules such as DNA, lipids and proteins (Breimer 1990; Romert et al 1998; Souza et al, 1999); that results in inactivation of antioxidant enzymes (Pigeolet et al, 1990). Nearly all of the energy needed by the human body is provided by the oxidation of carbohydrates and lipids. Whereas carbohydrates provide a readily available source of energy, lipids function primarily as an energy reserve.

Cassava is a staple food in human diets in over 80 countries (Gomez, et al 1988). Gari a starchy food prepared from cassava (*Manihot utilisima*) tubers is one of the most popular staple foods of the people of the rain forest belt of West Africa and contains mainly starch-20% amylase and 70% amylopectin having lost the soluble carbohydrates (i.e. glucose and sugar) during processing.). Gari is rich in starch. It also has very high fibre content and also contains proteins and some essential vitamins. Gari diet has been shown to reduce enzymes induced by petroleum hydrocarbon (Braide, *et al* 2011).

The aim of this paper was to establish a possible protective role of gari diet against petroleum induced

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change in lipids using cholesterol, triglycerides, HDL and LDL cholesterol as indicators.

MATERIALS AND METHODS

Test Animals : Ninety Wistar albino rats of 0.195kg average body weight on normal rat diet were obtained from the animal house of the department of Pharmacology and Toxicology, University of Port Harcourt. These rats were fed adlibitum with normal rat pellet and water and acclimatized to laboratory conditions for a period of 14days prior to commencement of study. The gari used in this study was purchased from Mile 3 Market, Port Harcourt. The crude petroleum used (Bonny Light) was obtained from the Nigerian National Petroleum Corporation (N.N.P.C.) Zonal Office at Moscow Road, Port Harcourt. Commercially prepared Cholesterol, triglycerides, and HDL precipitant were obtained from Randox Diagnostics, London.

Animal Studies: Preliminary study was done to ascertain the oral LD_{100} and LD_{50} of crude oil. Preliminary study was also done by authors to ascertain the gari concentration that will cause reduction in cholesterols by feeding rats with various concentrations of gari and observing the concentration of gari with the lowest cholesterol level.

The gari treated albino rats were fed diet contaminated with crude oil at concentrations of 3.88, 7.75, 15.51, 31.01 and 62.02g/kg (of crude oil) mixed with 20% gari while the last group was fed rat diet with distilled water adlibitum. The petroleum treated albino rats were fed diet contaminated with crude oil at concentrations of 3.88, 7.75, 15.51, 31.01 and 62.02g/kg (of crude oil) while the last group was fed rat diet with distilled water adlibitum to serve as control. Preliminary investigation had established that this concentration of crude oil was tolerable to the albino rats on a prolonged basis without any drastic effect.

Biochemical Studies: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxides (Allain et al 1974)

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Ten microlitre (10 μl) of sample, control, standard and distilled water was pipette into respective test tube then 1000 μl of cholesterol working reagent was added. It was mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample was measured against the reagent blank at 520nm. The concentration of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard.

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase (Buccolo and David 1973).

Ten microlitre (10) μl of sample, control, standard and distilled water was pipetted into respective test tube then 1000 μl of triglyceride reagent was added. It was mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample was measured against the reagent blank at 520nm. The concentration of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard.

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, was determined.

Five hundred (500) μl of sample, control standard and distilled water was added into respective test tubes, 1000 μl of precipitant was added into all the tubes. It was mixed and allowed to stand for 10 minutes at room temperature. It was centrifuged for 2 minutes at 12,000 rpm. Then 10 μl of supernatant from control, standard and distilled water was added into their respective test tubes and cholesterol concentration of supernatant was determined as shown above by method of Allain et al (1974).

LDL-cholesterol was calculated using the formula of Friedwald et al (1972) as shown below

LDL-cholesterol (Mmol/L)=Total cholesterol (Mmol/L)-(HDLC (Mmol/L)+TG/2.22)(Mmol/L).

RESULT AND DISCUSSION

There were doses dependent increase in cholesterol and LDL cholesterol concentrations (Mmol/L), with dose dependent decrease in triglycerides and HDL cholesterol concentrations in petroleum treated albino rats compared with their controls. The gari treated albino rats had dose dependent decrease in cholesterol and LDL cholesterol concentrations (Mmol/L), with dose dependent increase in triglycerides and HDL cholesterol concentrations compared with petroleum treated albino rats. The

Cholesterol concentration (Mmol/L) of the control in Petroleum treated albino rats was 2.66 ±0.12. At 3.88g/kg of petroleum treatment, the concentration was 2.79 ±0.16, while it increased to 3.30 ±0.13, 3.26 ±0.08, 3.68 ±0.07 and 3.75 ±0.09 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The cholesterol concentration for control in gari treated albino rats was 2.62 ±0.17. At 3.88g/kg of gari treatment, concentration was 2.64 ±0.29, while it increased to 3.11 ±0.11, 3.13±0.28, 3.15 ±0.28 and 3.22 ±0.36 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown below in table 1.

The triglyceride (Mmol/L) of 1.36±0.14 was obtained in the control of petroleum treated albino rats which reduced to 1.11 ± 0.03 at 3.88g/kg. The concentrations further increased to 1.11 ±0.01, 1.02 ± 0.02, 0.91±0.01, and 0.87± 0.01 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The triglyceride concentration of 1.34±0.11 was obtained in the control of gari treated albino rats which reduced to 1.25 ±0.04 at 3.88g/kg. The concentration further decreased to 1.06 ± 0.03, 1.03±0.01, 1.02± 0.07 and 0.96 ± 0.02 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown below in table 1. Ben-David et al (2001) submitted that the ingestion of petroleum caused reduction in blood glucose. This may shift the demand for metabolic substrate to lipid, thus a significant decrease in the level of triglycerides in rats fed petroleum contaminated diet relative to control animals. Increase in the metabolism of lipids has been reported to induce generation of free radicals (Patockova *et al* 2003). Achuba (2005) also reported increase cholesterol level in rabbits fed crude oil contaminated diet which was reversed by feeding with antioxidant vitamins E and C. In this study feeding 20% gari reversed the induced Cholesterol.

TABLE 1 Effect Of Gari On Cholesterol And Triglycerides In Albino Rats Treated With Petroleum

	CHOLESTEROL (Mmol/L)			**TRIGLYCERIDES (Mmol/L)**		
Concentration (g/kg)	Petroleum treated	Gari Treated	P value	Petroleum treated	Gari Treated	P value
0.00	2.66 ±0.12	2.62 ±0.17	0.893	1.36±0.14	1.34±0.11	0.929
3.88	2.79 ±0.16	2.64 ±0.29	0.476	1.11 ± 0.03	1.25 ±0.04	0.007
7.75	3.30 ±0.13	3.11 ±0.11	0.392	1.11 ±0.01	1.06 ± 0.03	0.139
15.51	3.26 ±0.08	3.13±0.28	0.717	1.02 ± 0.02	1.03±0.01	0.444
31.01	3.68 ±0.07	3.15 ±0.28	0.097	0.91±0.01	1.02± 0.07	0.109
62.02	3.75 ±0.09	3.22 ±0.36	0.186	0.87± 0.01	0.96 ± 0.02	0.004

TABLE 2 Effect Of Gari On HDL And LDL Cholesterols In Albino Rats Treated With Petroleum

	HDL CHOLESTEROL (Mmol/L)			LDL CHOLESTEROL (Mmol/L)		
Concentration (g/kg)	Petroleum treated	Gari Treated	P value	Petroleum treated	Gari Treated	P value
0.00	2.58±0.21	2.42 ± 020	0.461	1.43 ± 0.41	1.25 ± 0.33	0.806
3.88	1.94 ±0.07	2.08 ±0.10	0.334	0.28 ± 0.09	0.58 ± 0.17	0.108
7.75	1.91 ±0.11	1.84 ± 0.01	0.610	0.73 ± 0.20	0.61 ± 0.10	0.692
15.51	1.85 ± 0.04	1.88 ± 0.01	0.437	0.93 ± 0.10	0.74 ± 0.30	0.644
31.01	1.77 ± 0.03	1.82 ±0.02	0.305	1.63 ± 0.11	0.94 ±0.16	0.023
62.02	1.71 ± 0.01	1.81 ± 0.04	0.139	1.77 ± 0.02	1.28 ± 0.25	0.119

TABLE 3 Effect Of Gari On Lipid Concentration In Albino Rats Treated With Petroleum

PARAMETER (Mmol/L)	**PETROLEUM**	**GARI**	**T**	**P VALUE**
Cholesterol	3.3568 ± 0.08	3.0516 ±0.12	2.664	0.014
Triglycerides	1.0020 ±0.02	1.06 ± 0.03	-2.473	0.021
HDL cholesterol	1.84 ± 0.03	1.89 ± 0.03	-1.399	0.175
LDL cholesterol	1.07 ±0.12	0.83 ± 0.10	2.086	0.048

The HDL Cholesterol concentration (Mmol/L) of the control in Petroleum treated albino rats was 2.58±0.21. At 3.88g/kg of petroleum treatment, the concentration was 1.94 ±0.07, while it decreased to 1.91 ±0.11, 1.85 ± 0.04, 1.77 ± 0.03 and 1.71 ± 0.01 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The HDL cholesterol concentration for control in gari treated albino rats was 2.42 ± 020. At 3.88g/kg of gari treatment, concentration was 2.08 ±0.10, while it reduced to 1.84 ± 0.01, 1.88 ± 0.01, 1.82 ±0.02 and 1.81 ± 0.04 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown below in table 2.

The LDL Cholesterol concentration (Mmol/L) of the control in Petroleum treated albino rats was 1.43 ± 0.41. At 3.88g/kg of petroleum treatment, the concentration was 0.28 ± 0.09, while it increased to0.73 ± 0.20, 0.93 ± 0.10, 1.63 ± 0.11 and 1.77 ± 0.02 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The LDL cholesterol concentration for control in gari treated albino rats was 1.25 ± 0.33 At 3.88g/kg of gari treatment, concentration was 0.58 ± 0.17, while it increased to 0.61 ± 0.10, 0.74 ± 0.30, 0.94 ±0.16 and 1.28 ± 0.25 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown below in table 2. The role of free radicals in the pathogenesis of antherosclerosis via oxidation of low-density lipoprotein that damage the arterial walls have been recognised (Harman, 1992). The ingestion of crude petroleum contaminated diet imposed a reciprocal relationship between HDL-cholesterol and LDL-cholesterol in the plasma of albino rats. The decrease in HDL-cholesterol with a corresponding increase in LDL-cholesterol is the primary risk factor for coronary heart disease (Mckee and Mckee, 1999; Glew, 1997). The supplementation of the diet with gari reduced the reduction in blood glucose hence the shift in demand for lipid will be reversed.

There was significant lowering of cholesterol concentration (Mmol/L) between 3.3568 ± 0.08 of Petroleum fed rats compared with 3.0516 ±0.12 of gari fed rats. Triglyceride (Mmol/L) concentration was increased from 1.0020 ±0.02 in petroleum treated rats by gari feeding to 1.06 ± 0.03. The HDL Cholesterol (Mmol/L) concentration of 1.84 ± 0.03 in petroleum treated rats was not significantly different from 1.89 ± 0.03 in gari treated albino rats. The LDL cholesterol (Mmol/L) of 1.07 ±0.12 in petroleum treated rats was significantly different from 0.83 ± 0.10 in gari treated rats as shown below in table 3. There was a significant increase in total cholesterol and LDL-Cholesterol. However an in significant (P>0.05) decrease in HDL-cholesterol and significant decrease triglycerides in animals fed petroleum contaminated diet relative to animals fed normal diet was observed. This is similar to study by Achuba (2005) and other authors (Onwurah, 1999; Shertzer *et al* 1994; Bronk and Gores, 1991; Khan *et al* 2001; Anozie, and Onwurah 2001). The supplementation of the diet with gari reduced the reduction in blood glucose hence the shift in demand for lipid will be reversed causing increase triglycerides.

Conclusion: It is pertinent to conclude that ingestion of petroleum contaminated diet could predispose humans to cardiovascular diseases. This study has shown that feeding on crude oil contaminated diets caused changes in lipid profile while feeding on gari reversed the changes by reducing the induced cholesterol and LDL cholesterol while triglyceride was increased due to shift in demand for lipids as substrate. The pretreatment of the feed with gari prior to the exposure to the experimental rats decrease the toxic effect of crude oil as exhibited by the restoration of lipid profile to control values as shown in this study.

REFERENCES

Achuba, F.I., (2005): Effect of vitamins C and E intake on blood lipid Concentration, lipid peroxidation, superoxide dismutase and catalase activities in Rabbit fed petroleum contaminated diet. *European Journal of Scientific Research,* **12** (1): 20-28.

Achuba F.I. and Osakwe S.A. (2003). Petroleum induced free radical toxicity in African catfish (Clarias gariepinus) *Fish Physiol Biochem* 29(2):97-103.

Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W.and Fu, P.C. (1974).Enzymatic determination of total serum cholesterol. *Clinical Chemistry,* 20: 470-3

Amadi A, Dickson A A and Maate (1993). Remediation of Oil polluted soils: 1 Effect of organic and inorganic nutrient supplemention the performance of maize (Zea Mays). *Water, Air Soil Pollution,* 66:59-76.

Anozie, O. I., and Onwurah, I. N. E., (2001): Toxic effect of Bonny light Crude oil in rat after ingestion of contaminated diet. *Nigeria Journal of Biochemistry and Molecular Biology* (Proc. Suppl), **16**:103s – 108s.

Ben-David, M, Duffy L.K and Bowyer RT (2001) Biomarker responses in river otters experimentally exposed to oil contamination. *Journal of Wildlife Disease,* 37 (3): 489 – 508.

Braide, A. S., Adegoke, O. A., Bamigbowu, E. O. (2011). Effect of Cassava based diet on hepatic proteins in albino rats fed with crude oil contaminated diet. *Journal of Applied Science and Environmental Management* 15(1):223-229.

Breimer L.H. (1990). Moleculer mechanisms of oxygen radical caranogenesis and mutagenesis: the role of DNA base damage. *Mol Carcinogenesis* 3:188-197.

Bronk S.F and Gores G.F (1991) Acidosis Protects against lethal injury of liver simusoidal endothelial cell. *Hepathology* 14:150-157.

Buccolo, G. and David, H. (1973). Quantitative determination of serum triglycerides by the use of Enzymes. *Clinical Chemistry* 19:476-482.

Coppock, R. W., Mostrom, M.S., Khan, A., Semalula, S.S. (1995): Toxicology of oil field pollutants in cattle: A review. *Veterinary and Human Toxicology,* **37** (6): 369 – 576.

Friedwald, T.W, Fredrickson, D.S. and Levy, R.J. (1972). LDL cholesterol estimation. *Clinical Chemistry* 18:499-501.

Glew RH (1997) Lipid Metabolism 11: pathways of metabolisms of special lipids. In *textbook of Biochemistry with clinical correlations* Devlin TM (ed) 4th edition. Wiley-Liss In C New York pp 415-417.

Gomez, G., Aparicho, M.A. and Willhite, C.C.(1988). Relationship between dietary cassava cyanide levels and brailer performance. *Nutrition Report International*; **37**:63-75.

Halliwell, B (1994). Free radicals, antioxidants and human disease: Curiosity, cause or consequence? *Lancet* 334:721-724.

Harman, D (1992) Role of free radicals in aging and disease. *Annal New York Academy of Science* 673:126-141.

Khan, A. A., Coppock, R. W. and Schuler, M. M., (2001): Effect of multiple exposures of small doses of pembina cardium crude oil and diesel in rats. *Archive of Environmental Contamination and Toxicology,* **40**:418 – 424.

Mckee, T and Mckee, RI (1999) *Biochemistry* 2nd Ed McGraw Hill, New York pp 135- 238

Onwurah, INE (1999) Lipid peroxidation and protein oxidation in Azotobacter vinelandii exposed to mercury, silver, crude oil and Fenton reagent. *Journal of Toxic substance,* 18(4) 167-176.

Patockova J, Marhol P. Tumova E, Krsiak M Rokyta R, Stipek S, Crkovska J and Andel M (2003). Oxidative stress in the brain tissue of laboratory mice with acute post insulin hypoglycemia. *Physiological Research,* 52:131-135.

Pigeolet, E, Corbisler P, Houbion A, Lamber D Michiels C (1990). Glutathione peroxidases, superoxide dismutase and catalase inactivation by peroxides and oxygen derived free radicals. *Mechanism Ageing Development,* 51:283-297.

Romero F.J., Bosch-Morell F., Romero M.J., Jareno E.J., Marine N. (1998). Lipid peroxidation products and antioxidant in human disease. *Environmental Health Perspectives,* 106 (Suppl) 5:1390-1393.

Shertzer H.G, Bannenberger G K, Zhu H, Liu R M and Moildeus P (1994). The role of thiol in mitchondrial susceptibility to iron and tert-butyl hydroperoxide mediated toxicity in cultured mouse hepatocytes. *Chemical Research of Toxicology,* 7:358-366.

Souza, M. F., Tome A. R. and Rao V. S. (1999). Inhibition by the bioflavonoid ternation on Aflatoxin B1-idncued lipid peroxidation in rat liver. *J. Pharm Pharmacol* 51:125-129.

Val A.L and Almeida-Val V.F (1999). Effect of crude oil on respiratory aspect of some fish species of the Amazon. In Val AL and Almeida-Val V MF (eds) *Biology of Tropical Fish*, Manaus Brasil. Pp 227-291.

Volkman, J. K.; Miller, G. J.; Revill, A. T.; Connell D. W.(1994). Oil spills. In: *Environmental Implications of offshore oil and Gas Development in Australia - The findings of an Independent Scientific Review* (Swan J. M., Neff J. M., and Young P. C., Eds) *Australian Petroleum Exploration Association, Sydney,* Pp.509-695.