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Comparative Study of the Protective Effect of Granulated Sugar and Garri Meal on Petroleum-induced Changes of Lipid Profiles in Albino Rats (*Rattusnorvegicus*)

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ABSTRACT: Crude oil contaminated diet has been reported to induced changes in lipid profile of rats. In this study, 60 albino rats in 6 groups were fed with crude oil contaminated sugar-garri diets at varied concentrations to monitor petroleum-induce lipid profile changes using standard methods. The lipid profiles estimated were total cholesterols, triglycerides, high density lipoprotein cholesterol and Low density lipoproteincholesterol. The data showed that, the levels of total cholesterol (Mmol/l) increased from 2.89+0.08 in sugar fed rats compared with 3.05+0.12 in Gari fed rats. The concentration of triglycerides(Mmol/l) reduced from 1.10 ± 0.51 in sugar fed rats to 1.06 ± 0.03 in Gari fed rats while the high density lipoprotein cholesterol(Mmol/l) concentration of 2. 01+0.08 was significantly higher than 1.89 ± 0.03 in Gari fed rats (P<0.05). There was no significant difference in Low density lipoprotein cholesterol (Mmol/l) concentrations of 0.68 ± 0.11 in sugar fed rats compared with 0.83 + 0.10 in Gari fed rats. The effect of pre-treatment of the diet with sugar reduced petroleum-induce changes in lipid profile than gari during the exposure of the albino rats to the petroleum diet suggesting that sugarcould possibly ameliorate petroleum-induce changes in lipid profile as shown by the significantlowering of the cholesterol and Low density lipoprotein cholesterol than gari in albino rats. © JASEM https://dx.doi.org/10.4314/jasem.v21i2.12

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Crude oil has been described as a complex mixture of over 6000 potentially different hydrocarbons and metal (Edwards, 1989) and the chemical composition varies between geologic formations (Coppock et al., 1995). Exposure of humans and animal to crude oil, which is increasing in terms of the environmental levels, and application to body, may be toxic. Crude oil is used in folkloric medicine in the Niger-delta area of Nigeria for the treatment of various ailments including stomach up-set, wound, and burns (Orisakwe, et al., 2000). The route of administration is mostly oral and external application for burns and wounds. 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 macromolecules such as DNA, lipids and proteins (Breimer 1990; Romero et al 1998; Souza et al, 1999); that results in inactivation of antioxidant enzymes (Pigeolet et al., 1990). Hydrocarbons and other constituents of petroleum and petrochemical products like other xenobiotics are metabolized in the liver to a greater extent (Sims, 1980). Achuba (2005) had reported that total cholesterol and LDLcholesterol were insignificantly increased while HDL-cholesterol and triglyceride significantly decreased in rabbits fed petroleum contaminated diet and the ingestion of crude petroleum contaminated diet imposed a reciprocal relationship between HDLcholesterol and LDL-cholesterol in the plasma of Rabbit. Nearly all of the energy needed by the human body is provided by the oxidation of carbohydrates and lipids.

The sugar cane is one of the most important sucrose sources, containing until 20% weight sucrose (Glazer and Nikaido, 1995). Sucrose hydrolysis produces a fructose and glucose equimolar mixture named inverted sugar, which has higher edulcorant power. The inverted sugar is incorporated more easily in industrial preparations and has more added value than sucrose (Chou and Jasovsky 1993). Sugar has been reported to reduce haematoxicity caused by petroleum (Braide *et al.*, 2011a).

Cassava was transplanted from Brazil to Africa by the Portuguese nearly three centuries ago (Bourdoux et al., 1980). Gari processing consisted of peeling, shredding, fermenting and dehydrating (light roasting) in sacks for 3-4 d, sifting, gelatinizing and roasting. Gari is made by grating cassava (Mannihot esculenta Crantz) and then dehydrating the fibre in sacks for 3 days after which the residue is roasted. Cassava is a staple food in human diets in over 80 countries (Gomez et al., 1988). Gari a starchy food prepared from cassava (Mannihot esculenta *Crantz*)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 has been reported to reduce enzymes induction caused by petroleum through the phenomenon of glucose effect (Braide et al., 2011b).

The aim of this paper was to compare possible protective roles of sugar and gari diets against petroleum induced changes in lipids using cholesterol, triglycerides, high density lipoprotein cholesterol and Low density lipoproteincholesterol as indicators.

MATERIAL AND METHODS

Experimental animals: Sixty 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 and sugar 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 carried out by authors to ascertain the sugar and gari concentrations that will cause glucose effect by feeding rats with various concentrations of sugar and gari, then observing the concentration of sugar and gari with the lowest cholesterols concentration. The albino rats were fed contaminated diet mixed with 20% sugar and 20% gari adlibitum for three weeks, slaughtered and blood samples taken for analysis. The concentrations of crude oil used were 3.88, 7.75, 15.51, 31.01 and 62.02g/kg 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.

Biochemicalstudies: 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).

Ten microlitre $(10 \,\mu l)$ of sample, control, standard and distilled water was pipette into respective test tube then $1000 \,\mu l$ of cholesterol working reagent was added. It was mixed and incubated for 5 minutes at 37^{0} C. 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°C. 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).

Statistical analysis: The biochemical data were subjected to some statistical analysis. Values were reported as Mean \pm SEM while student's t-test was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 16.A value of P<0.05 was accepted as significant.

RESULTS AND DISCUSSION

The cholesterol concentration for control in sugar treated albino rats was 1.64 ±0.29. At 3.88g/kg of sugar treatment, concentration was 2.64 +0.22, while it increased to 2.74 ± 0.24 , 2.92 ± 0.16 , 3.06 ± 0.15 and 3.09 ± 0.96 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The Cholesterol concentration (Mmol/L) of the control in gari treated albino rats was 2.62 +0.17. At 3.88g/kg of gari treatment, the 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. One of the major problems of the inhabitants of the Niger Delta region of Nigeria is contamination of water and aquatic lives by crude oil. This contamination may not necessarily lead to outright mortality but may have significant effects which can lead to physiological stress and dysfunction in animals (Omoregie, 1998). The result showed a reduction in cholesterol concentration of sugar fed compared with gari fed. This suggests that feeding on 20%sugar tend to reduce cholesterol in petroleum treated rats than gari treated rats. Achuba (2005) had reported that feeding rabbits with diets containing

various concentrations of crude petroleum caused significant dose related increases in cholesterol suggesting that ingestion of petroleum contaminated diet could predispose humans to cardiovascular diseases while the result of this study has shown that sugar diet reduced cholesterol concentration more than gari diet. Adegoke et al. (2013) reported that feeding sugar and gari can cause synthesis of total and HDL cholesterols but the increase is more pronounced in gari than sugar. Braide et al (2011c) in their study reported that sugar diet reduced enzymes induction in petroleum treated rats more than gari. This was attributed to cyanogenic component present in gari. Ezeji et al (2009) had suggested that cyanide affects some important enzymes of the mitochondtrial electron transport system which are used as markers of the organelle.

The triglyceride concentration of 1.38+0.11 was obtained in the control of sugar treated albino rats which reduced to 1.28 ± 0.20 at 3.88 g/kg. The concentration further decreased to 1.15 + 0.01, 1.03 ± 0.16 , 1.03 ± 0.03 and 1.01 ± 0.01 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The triglyceride (Mmol/L) 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 concentrations further increased 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. Achuba (2005) reported significantly decreased triglyceride in rabbits fed petroleum contaminated diet but the result of this study showed that sugar fed rats increased triglycerides more than the gari fed rats. In humans, hypertriglyceridemia can be induced endogenously by a high fat diet (Austin et al., 1998) or it can be carbohydrate induced (Knittle and Ahrens 1964, Reavenet al 1965). A high triacylglycerol (TAG) flux rate was observed in subjects fed HCLF diet (Abbott, et al., 1990). The TAG increase observed in humans on a HCLF diet may have been from de novo lipogenesis (Parks, and Hellerstein 2000). Ben-David et al (2001) reported that the ingestion of petroleum caused reduction in blood glucose causing shift in the demand for metabolic substrate to lipid, the supplementation of the diet with sugar and gari reversed the reduction in blood glucose hence the shift in demand for lipid will be reversed causing increase triglycerides. The increase in triglycerides concentrations of sugar more than gari might be as a result of some other components like cyanogens present in gari.

	CHOLESTEROL (Mmol/L)			TRIGLYCERI		
CONCENTRATION	SUGAR	GARI	P VALUE	SUGAR	GARI	P VALUE
(g/kg)	TREATED	TREATED		TREATED	TREATED	
0.00	2.64 ± 0.29	2.62 <u>+</u> 0.17	0.337	1.38 ± 0.11	1.34 <u>+</u> 0.11	0.911
3.88	2.64 ± 0.22	2.64 <u>+</u> 0.29	0.473	1.28 ± 0.20	1.25 <u>+</u> 0.04	0.703
7.75	2.74 ± 0.24	3.11 <u>+</u> 0.11	0.006	1.15 ± 0.01	1.06 <u>+</u> 0.03	0.163
15.51	2.92 ± 0.16	3.13 <u>+</u> 0.28	0.006	1.03 ±0.16	1.03 <u>+</u> 0.01	0.473
31.01	3.06 ± 0.15	3.15 <u>+</u> 0.28	0.163	1.03 ± 0.03	1.02 <u>+</u> 0.07	0.473
62.02	3.09 ± 0.96	3.22 <u>+</u> 0.36	0.703	1.01 ± 0.01	0.96 <u>+</u> 0.02	0.275

Table 1 Effect of Gari and Sugar on cholesterol and triglycerides in albino rats treated with petroleum

The HDL cholesterol concentration for control in sugar treated albino rats was 2.46 ± 0.03 at 3.88 g/kg of sugar treatment; concentration was 2.30 +0.08, while it reduced to 2.07 + 0.02, 1.90 + 0.03, 1.91 ± 0.03 and 1.88 ± 0.03 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The HDL Cholesterol concentration (Mmol/L) of the control in gari treated albino rats was 2.42 + 020. At 3.88g/kg of gari treatment, the concentration was 2.08 +0.10, while it decreased 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 for control in sugar treated albino rats was 1.46 + 0.14. At 3.88g/kg of sugar treatment, concentration was 1.06 + 0.37, while it reduced to 0.40 + 0.15, 0.60 \pm 0.05, 0.62 \pm 0.18 and 0.73 \pm 0.11 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The LDL Cholesterol concentration (Mmol/L) of the control in gari treated albino rats was 1.25 ± 0.33 . At 3.88g/kg of gari treatment, the concentration was 0.58 ± 0.17 , while it increased to 0.61 ± 0.10 , 0.74 + 0.10 $0.30, 0.94 \pm 0.16$ 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. This study reported an increase HDL cholesterol with reduction in LDL cholesterol concentrations of sugar fed albino rats compared to gari fed rats suggesting that sugar diets increases good cholesterol as HDL is known while reducing the LDL cholesterol than gari diet. Achuba (2005) had reported that the ingestion of crude petroleum contaminated diet imposed a reciprocal relationship between HDL-cholesterol and LDLcholesterol in the plasma of Rabbit. 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). Achuba (2005) reported that supplementation of diet with vitamins E and C could possibly ameliorate petroleum-induce changes in lipid profile while vitamin E appeared more effective than vitamin C

Table 2 Effects of gari and sugar on HDL cholesterol and LDL cholesterol in albino rats treated with petroleum

	HDL CHOLESTEROL (Mmol/L)			LDL CHOLESTEROL (Mmol/L)		
CONCENTRATION	SUGAR	GARI	P VALUE	SUGAR	GARI	P VALUE
(g/kg)	TREATED	TREATED		TREATED	TREATED	
0.00	2.46 ± 0.03	2.42 <u>+</u> 0.20	0.862	1.46 ± 0.14	1.25 <u>+</u> 0.33	0.911
3.88	2.30 ± 0.08	2.08 <u>+</u> 0.10	0.911	1.06 ± 0.37	0.58 <u>+</u> 0.17	0.044
7.75	2.07 ± 0.02	1.84 <u>+</u> 0.01	0.401	0.40 ± 0.15	0.61 <u>+</u> 0.10	0.980
15.51	1.90 ± 0.03	1.88 <u>+</u> 0.01	0.05	0.60 ± 0.05	0.74 <u>+</u> 0.30	0.401
31.01	1.91 ± 0.03	1.82 <u>+</u> 0.02	0.05	0.62 ± 0.18	0.94 <u>+</u> 0.16	0.499
62.02	1.88 ± 0.03	1.81 <u>+</u> 0.04	0.05	0.73 ± 0.11	1.28 <u>+</u> 0.25	0.674

There was significant lowering of cholesterol concentration (Mmol/L) in sugar treated albino rats with 2.89±0.08 compared with 3.05±0.12 of gari fed rats. Triglyceride (Mmol/L) concentration was increased from 1.10 ± 0.51 in sugar treated rats compared with 1.06 ± 0.03 in gari treated rats. The HDL Cholesterol (Mmol/L) concentration of 1.89 ± 0.03 in gari treated rats was significantly lower than 2.01 ± 0.08 in sugar treated albino rats. The LDL cholesterol (Mmol/L) of 0.83 ± 0.10 in gari treated rats was significantly different from 0.68 ± 0.11 in sugar treated rats as shown below in table 3.Braide*et al.*, (2013) in their study reported that ingestion of petroleum contaminated diet caused increase cholesterol and LDL cholesterol and decreased

triglycerides and HDL cholesterol while feeding on gari diet caused reversed to changes in lipid concentration caused by crude petroleum. Also Adegoke *et al.*, (2012) reported that ingestion of petroleum contaminated diet caused decreased haemoglobin (Hb), Packed cell volume (PCV) and white blood cell count (an indicator of possible blood damage) but supplementation of the diet with 20% gari further decreased the haemoglobin (Hb), Packed cell volume (PCV) and white blood cell count observed in petroleum fed albino rats.Therefore, this study has shown that sugar diet appeared to ameliorate petroleum-induced changes in lipid profile compared with gari. This may as a result of

cyanogens present in gari which is absent in granulated sugar used in this study.

Table 3 effect of Sugar and Gari on lipid concentration in albino rats treated with petroleum

concentration in aromo rats treated with perioreum						
Sugar	Gari	P value				
2.89 <u>+</u> 0.08	3.05 <u>+</u> 0.12	0.063				
1.10 <u>+</u> 0.51	1.06 <u>+</u> 0.03	0.088				
2.01 <u>+</u> 0.08	1.89 <u>+</u> 0.03	0.040				
0.68 <u>+</u> 0.11	0.83 <u>+</u> 0.10	0.437				
	Sugar 2.89 <u>+</u> 0.08 1.10 <u>+</u> 0.51 2.01 <u>+</u> 0.08	Sugar Gari 2.89±0.08 3.05 ±0.12 1.10±0.51 1.06 ± 0.03 2.01±0.08 1.89 ± 0.03				

*Conclusion:*This study has shown that sugar diet appeared to ameliorate petroleum-induced changes in lipid profile compared with gari. The effect of pre-treatment of the diet with sugar reduced petroleum-induce changes in lipid profile than gari during the exposure of the albino rats to the petroleum diet suggesting that sugar could possibly ameliorate petroleum-induce changes in lipid profile as shown by the significant lowering of the cholesterol and Low density lipoprotein cholesterol than gari in albino rats.

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