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 dependant 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 macromolecules 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 ad libitum with normal rat pellet and water and acclimatized to laboratory conditions for a period of 14 days 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 cholesterol 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 ad libitum. 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 ad libitum 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 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$\mu$l) of sample, control, standard and distilled water was pipetted into respective test tubes then 1000 $\mu$l 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)$\mu$l of sample, control standard and distilled water was added into respective test tubes, 1000 $\mu$l 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 $\mu$l 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

\[ \text{LDL-cholesterol (Mmol/L)} = \text{Total cholesterol (Mmol/L)} - (\text{HDL cholesterol (Mmol/L)} + \text{TG/2.22}) \]

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

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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

<table>
<thead>
<tr>
<th>Concentration (g/kg)</th>
<th>CHOLESTEROL (Mmol/L)</th>
<th>P value</th>
<th>TRIGLYCERIDES (Mmol/L)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum treated</td>
<td>Gari Treated</td>
<td></td>
<td>Petroleum treated</td>
<td>Gari Treated</td>
</tr>
<tr>
<td>0.00</td>
<td>2.66 ±0.12</td>
<td>0.893</td>
<td>1.36±0.14</td>
<td>1.34±0.11</td>
</tr>
<tr>
<td>3.88</td>
<td>2.79 ±0.16</td>
<td>0.476</td>
<td>1.11 ±0.03</td>
<td>1.25±0.04</td>
</tr>
<tr>
<td>7.75</td>
<td>3.30 ±0.13</td>
<td>0.392</td>
<td>1.11 ±0.01</td>
<td>1.06±0.03</td>
</tr>
<tr>
<td>15.51</td>
<td>3.26 ±0.08</td>
<td>0.717</td>
<td>1.02 ±0.02</td>
<td>1.03±0.01</td>
</tr>
<tr>
<td>31.01</td>
<td>3.68 ±0.07</td>
<td>0.979</td>
<td>0.91±0.01</td>
<td>1.02±0.07</td>
</tr>
<tr>
<td>62.02</td>
<td>3.75 ±0.09</td>
<td>0.186</td>
<td>0.87±0.01</td>
<td>0.96±0.02</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration (g/kg)</th>
<th>HDL CHOLESTEROL (Mmol/L)</th>
<th>P value</th>
<th>LDL CHOLESTEROL (Mmol/L)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum treated</td>
<td>Gari Treated</td>
<td></td>
<td>Petroleum treated</td>
<td>Gari Treated</td>
</tr>
<tr>
<td>0.00</td>
<td>2.58±0.21</td>
<td>0.461</td>
<td>1.43±0.41</td>
<td>1.25±0.33</td>
</tr>
<tr>
<td>3.88</td>
<td>1.94±0.07</td>
<td>0.334</td>
<td>0.28±0.09</td>
<td>0.58±0.17</td>
</tr>
<tr>
<td>7.75</td>
<td>1.91±0.11</td>
<td>0.610</td>
<td>0.73±0.20</td>
<td>0.61±0.10</td>
</tr>
<tr>
<td>15.51</td>
<td>1.83±0.04</td>
<td>0.437</td>
<td>0.93±0.10</td>
<td>0.74±0.30</td>
</tr>
<tr>
<td>31.01</td>
<td>1.77±0.03</td>
<td>0.305</td>
<td>1.63±0.11</td>
<td>0.94±0.16</td>
</tr>
<tr>
<td>62.02</td>
<td>1.71±0.01</td>
<td>0.139</td>
<td>1.77±0.02</td>
<td>1.28±0.25</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter (Mmol/L)</th>
<th>Petroleum</th>
<th>Gari</th>
<th>T</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>3.35±0.08</td>
<td>3.05±0.12</td>
<td>2.664</td>
<td>0.014</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.0020 ±0.02</td>
<td>1.06±0.03</td>
<td>-2.473</td>
<td>0.021</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.84±0.03</td>
<td>1.89±0.03</td>
<td>-1.399</td>
<td>0.175</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>1.07±0.12</td>
<td>0.83±0.10</td>
<td>2.086</td>
<td>0.048</td>
</tr>
</tbody>
</table>

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 ±0.02. 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

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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 atherosclerosis 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

**REFERENCES**


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