Middle-East Journal of Scientific Research 19 (12): 1658-1663, 2014
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2014.19.12.11422

Effect of Cassava Based Diet on Renal Function in Crude Petroleum Treated Albino Rats

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Abstract: The study was carried out to ascertain the effect of a cassava based diet (gari) on renal functions in albino rats fed crude oil contaminated diets. Rat diet contaminated with various concentrations of crude oil mixed with 20% gari were fed albino rats to determine the effect of gari on the likely renal dysfunction caused by petroleum. The renal parameters (Creatinine, Urea, Sodium and Potassium) were monitored in the animals. There were dose dependent increases in potassium, urea and creatinine concentrations with dose dependent decrease in sodium concentration in both petroleum and gari fed rats compared with their respective controls. There was significant increase ($P<0.05$) in urea concentration of gari fed rats compared with petroleum fed rats. The presence of cyanide may account for the increase urea in the gari fed causing defective renal glomerular filtration. Therefore this study showed that feeding on petroleum causes renal dysfunction while supplementation of the diet with gari (a cassava based diet) caused further increase in urea due to the presence of cyanide.

Key words: Cassava · Renal Crude petroleum

INTRODUCTION

Glucose represses the induction of inducible operons by inhibiting the synthesis of cyclic Adenosine monophosphate (cAMP) a nucleotide that is required for the initiation of transcription of a large number of inducible enzyme systems including the Lac operon. Cyclic AMP (cAMP) is required to activate an allosteric protein called catabolite activator protein (CAP) which binds to the promoter CAP site and stimulates the binding of RNA polymerase to the promoter for the initiation of transcription, but cAMP must be available to bind to CAP which binds to DNA to facilitate transcription. In the presence of glucose, adenylase cyclase (AC) activity is blocked. AC is required to synthesize cAMP from ATP. Therefore if cAMP levels are low, CAP is inactive and transcription does not occur. In the absence of glucose, cAMP levels are high, CAP is activated by cAMP and transcription occurs (in the presence of lactose). Cassava is a staple food in human diets in over 80 countries [1]. 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

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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 [2].

Crude oil has been described as a complex mixture of over 6000 potentially different hydrocarbons and metal [3]. Crude petroleum contains hundreds of compounds and the chemical composition varies between geologic formations [4]. Most of the substances contained in crude petroleum occur naturally due to their presence in rock formation or in saltwater deposits from which the crude oil was drawn [5]. They have also been grouped into types as light, medium (Intermediate) and heavy depending on their density, physical and chemical properties.

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 [6]. The route of administration is mostly oral and external application for burns and wounds. The kidney can suffer considerable damage before losing sufficient function to modify the normal clinical indication of renal disease such as the serum creatinine concentration [7]. Approximately 50% or more of renal capacity can be lost before serum creatinine become abnormal and disease is detectable clinically. A battery made up of a combination of different types of test can aid in the detection of damage by a nephrotoxin and also allows for the determination of various threshold damages. The detection of renal damage at a reversible stage is necessary before effective preventive measures can be taken to halt the progress of damage to the irreversible stage.

The aim of this study is to determine the effect of cassava based diet (Gari) on renal function in albino rats fed crude petroleum hydrocarbon using sodium (Na), Potassium (K), Urea (Ur) and Creatinine (Cr) as indicator.

MATERIALS AND METHODS

Petroleum: 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.

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 Urea and Creatinine reagents 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 potassium, urea and creatinine concentrationns by feeding rats with various concentrations of gari and observing the concentration of gari with the lowest potassium, urea and creatinine levels.

Experimental Design: 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 sodium and potassium estimation was done using Flame Photometric method [8] sssss.

Urea estimation was done by Urease - Berthelot colorimetric method. Ten [10] microlitre of sample, standard, control and distilled water was pipette into test tube labeled sample, standard control and blank respectively. Hundred (100) microlitre of urea reagent 1 was added to all the tubes and incubated at 37°C for 10 minutes. 250 microlitres of urea solutions 2 and 3 was added to all the tubes, mixed and incubated at 37°C for 15 minutes. The absorbance of the sample, control and standard were read at 546nm against the content of the blank tube. The activity of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard [9].

Creatinine estimation was done by Jaffe's colorimetric method. Five hundred (500) millilitre of sample, standard, control and distilled water was pipette into test tube labeled sample, standard control and blank respectively containing five hundred (500) millilitre of trichloroacetic acid (TCA).The contents were mixed and spun at 2500rpm for 10minutes. 1000 millilitre of supernatant from each tube was added into respectively labeled test tube containing 1000 millilitre of reagent mixture of Picric acid and sodium hydroxide (500 millilitre each). The contents were mixed and stand at 25°C for 20 minutes. The absorbance of the sample, control and standard were read at 546nm against the content of the blank tube. The concentration of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard [10].

Statistical Analysis: The biochemical data were subjected to some statistical analysis as the Mean (X), standard deviation (SD), standard error of mean (SEM) and student's t-test using Statistical Package for Social Sciences (SPSS) version 16.

RESULT

The sodium (Mmol/L) of control in Petroleum treated albino rats was 150.67±1.45. At 3.88g/kg of petroleum treatment, the sodium concentration was 145.33±0.88, while it reduced to 145.00 ± 3.51, 144.67 ± 2.03, 137.67 ± 1.86 and 136.33 ± 1.20 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The sodium (Mmol/L) of control in gari treated albino rats was153.00 ±10.44 At 3.88g/kg of gari treatment, the sodium (Mmol/L) was 148.67 ± 4.06, while it reduced to 143.33±5.46, 135.67 ± 2.84, 135.00 ±8.50and 129.00± 20.13. at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown below in Table 1.

The Potassium (Mmol/L) of control in Petroleum treated albino rats was5.13 ±0.19. At 3.88g/kg of petroleum treatment, the Potassium concentration was 5.23 ± 0.80, while it increased to 5.46 ± 0.26, 5.73 ±0.19, 6.30 ± 0.79 and6.47 ± 0.72 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The Potassium (Mmol/L) of control in gari treated albino rats was4.77 ± 0.26. At 3.88g/kg of gari treatment, the Potassium (Mmol/L) was 5.07 ± 0.80, while it increased to 5.13 ± 0.41, 6.23 ± 0.66, 6.30 ± 0.96 and 6.57± 0.58 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown below in Table 1.

The Urea (Mmol/L) of control in Petroleum treated albino rats was 6.35± 1.55. At 3.88g/kg of petroleum treatment, the urea concentration was 12.16±6.20. The other concentrations include 10.65± 1.26, 10.51 ± 4.64, 19.24 ± 9.23 and 32.93 ±15.81 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The urea (Mmol/L) of control in gari treated albino rats was 6.70± 0.27. At 3.88g/kg of gari treatment, the urea (Mmol/L) was 11.84±1.67, while it increased to 12.32 ± 1.84, 22.09 ± 6.42, 28.60 ± 2.76 and 45.72 ± 11.77 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown below in table 2. The Creatinine (mg/dL) of control in Petroleum treated albino rats was 8.43 ± 0.33. At 3.88g/kg of petroleum treatment, the Creatinine concentration was 8.76 ± 1.26, while it increased to 8.93 ± 0.73, 11.36 ± 0.71, 17.04 ± 1.68 and 29.78 ± 11.91 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The Creatinine (mg/dL) of control in gari treated albino rats was8.24 ± 0.96. At 3.88g/kg of gari treatment, the creatinine (mg/dL) was12.70 ± 1.15, while it increased to 12.87 ± 1.18, 15.86 ± 1.95, 15.47 ± 7.35 and 28.38 ± 0.33 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown below in Table 2.

Overall, there was no significant difference in 144.67 ± 2.07sodium (Mmol/L) concentration of Petroleum fed rats compared with 143.13 ± 3.54 of gari fed rats ($P>0.05$). Also Potassium (Mmol/L) concentration of 5.84 ± 0.24 in Petroleum treated rats was not significantly different from 5.86 ± 0.32 in gari treated rats ($P>0.05$). The Urea (Mmol/L) activity of 17.10 ± 4.27 in Petroleum treated rats was significantly different from 24.11 ± 6.25 in gari treated albino rats ($P<0.05$). The Creatinine concentration (mg/dL) of 15.18 ± 3.95 in petroleum treated rats was not significantly different from 17.06 ± 2.90 in gari treated rats ($P>0.05$).

DISCUSSION

The result obtained in this study showed dose dependent increase in Potassium, urea and creatinine with dose dependent decrease in sodium concentrations in both petroleum and gari fed albino rats. This is similar to the result of Orisakwe, *et al*. [11]. 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 [6]. The route of administration is mostly oral and external

Table 1: Effect of Gari on Sodium and Potassium in Rats fed Crude petroleum contaminated diets

	Sodium (Mmol/L)			Potassium (Mmol/L)		
Concentration (g/kg)	Petroleum Treated	Gari Treated	P Value	Petroleum Treated	Gari Treated	P Value
0.00	150.67±1.45	153.00 ±10.44	0.858	5.13 ±0.19	4.77 ± 0.26	0.386
3.88	145.33±0.88	148.67 ± 4.06	0.567	5.23 ± 0.80	5.07 ± 0.80	0.912
7.75	145.00 ± 3.51	143.33±5.46	0.497	5.46 ± 0.26	5.13 ± 0.41	0.603
15.51	144.67 ± 2.03	135.67 ± 2.84	0.188	5.73 ±0.19	6.23 ± 0.66	0.531
31.01	137.67 ± 1.86	135.00 ±8.50	0.729	6.30 ± 0.79	6.30 ± 0.96	1.000
62.02	136.33 ± 1.20	129..00± 20.13	0.737	6.47 ± 0.72	6.57± 0.58	0.936

Table 2: Effect of Gari on Urea and Creatinine in Rats fed Crude petroleum contaminated diets

	Urea (Mmol/L)			Creatinine (mg/dL)		
Concentration (g/kg)	Petroleum Treated	Gari Treated	P Value	Petroleum Treated	Gari Treated	P Value
0.00	6.35± 1.55	6.70± 0.27	0.866	8.43 ± 0.33	8.24 ± 0.96	0.868
3.88	12.16±6.20	11.84±1.67	0.965	8.76 ± 1.26	12.70 ± 1.15	0.244
7.75	10.65± 1.26	12.32 ± 1.84	0.553	8.93 ± 0.73	12.87 ± 1.18	0.166
15.51	10.51 ± 4.64	22.09 ± 6.42	0.394	11.36 ± 0.71	15.86 ± 1.95	0.116
31.01	19.24 ± 9.23	28.60 ± 2.76	0.334	17.04 ± 1.68	15.47 ± 7.35	0.874
62.02	32.93 ±15.81	45.72 ± 11.77	0.670	29.78 ± 11.91	28.38 ± 0.33	0.919

Table 3: Effect of Gari on renal parameters in Rats fed Crude petroleum contaminated diets

Parameter	Petroleum	Gari	T	P Value
Sodium (Mmol/l)	144.67 ± 2.07	143.13 ± 3.54	0.701	0.522
Potassium (Mmol/l)	5.84 ± 0.24	5.86 ± 0.32	-0.142	0.894
Urea (Mmol/l)	17.10 ± 4.27	24.11 ± 6.25	-3.014	0.039
Creatinine (mg/dL)	15.18 ± 3.95	17.06 ± 2.90	-0.338	0.752

application for burns and wounds. The kidney can suffer considerable damage before losing sufficient function to modify the normal clinical indication of renal disease such as the serum creatinine concentration [7]. The presence of cyanide in gari may be the reason for increase in potassium in gari treated than the petroleum treated. Ezeji *et al*. [12] reported that cyanide affects some enzymes of the mitochondrial electron transport system which are used as markers of the organelle and the rate of oxygen consumption is lowered upon prolonged gari feeding.

The result of this study also reported increase in creatinine and urea concentrations of both the petroleum and gari treated albino rats. This is similar to report of Ologunde *et al*. [13]. This may be due to the nephrotoxic effect of petroleum while the presence of cyanide in gari may also be the cause of further increase in the creatinine and urea concentrations in gari treated rats. Ologunde *et al*. [13] also reported that serum glucose, cholesterol, protein and creatinine levels were increased in animals fed Crude oil contaminated Cassava-based diet in spite of the level of processing. Ovuru, *et al*. [14] in their study using rabbit reported significant differences ($P < 0.05$) in biochemical parameters (urea, creatinine, total bilirubin, conjugated bilirubin and cholesterol) between control and crude oil treated animals indicating that ingestion of crude oil fractions cause disturbances in kidney and hepatocytes. Braide *et al*. [15] in their study suggested that feeding on 20% sugar diet may reverse the haemotoxic damage caused by crude petroleum as evidenced by increase in Hb and PCV concentrations possibly as result of glucose effect of sugar while sugar and gari were reported to reduce enzymes induction by reducing cAMP concentration [16].

Increased serum creatinine and lower creatinine clearances were related to diagnosis of chronic renal failure [17]. Creatinine is the anhydride of creatine. It is formed largely in the muscle by the irreversible and non enzymatic removal of water from creatine phosphate. Formation of creatinine is apparently a preliminary step required for the excretion of most of the creatine. Blood urea level is one of the routinely assessed markers of kidney function, but its reliability in assessment of kidney function is often compromised in the face of factors that significantly elevate it [18]. The central role played by the kidney in elimination of metabolic waste and the maintenance of pH balance cannot be contended [19].

Gari is a food made from cassava which is known to contain cyanide, a known inhibitor of the respiratory chain, the major source of ATP [20]. The processing

method for the gari may not eliminate effectively enough the cyanide present in the cassava [21]. Also the ability to detoxify cyanide may have been compromised due to the malnutrition. The presence of cyanide may account for the defective renal glomerular filtration, since mitochondrial electron transport has been negatively affected [12]. Olasore and Samuel [22] reported that during chronic protein-energy malnutrition due to consumption of poorly supplemented gari-based diet, certain renal functions are compromised. It may therefore be possible that the severity of renal dysfunction will not be as much when another carbohydrate rich food substance other than gari is consumed. Study has shown that granulated sugar caused changes in renal function based on the concentration of the granulated sugar [23].

CONCLUSION

This study has shown that feeding on gari contaminated with crude petroleum will cause damage to the kidney due to toxic effect of crude petroleum and cyanide present in gari.

ACKNOWLEDGEMENT

We wish to thank the Management of SIGMA laboratories, Port Harcourt for some of the analyses and Mr Enyaosa Tony for some literatures used in this study.

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