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Comparative Effects of Sugar and Gari Diets on Hepatic Enzymes in Rats Fed Crude Oil Contaminated Diet

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Abstract: The study was carried out to separately determine the effect of sugar and gari diet on albino rats fed crude oil contaminated diet by feeding albino rats diets contaminated with various concentrations of crude oil mixed with 20% sugar and 20%gari .The hepatic enzymes aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transpeptidase (GGT) and alkaline phosphatase (ALK PHOS) activities were monitored in the animals. There were dose dependent decrease in enzymes activities (ALT, AST, GGT and ALKPHOS) in Sugar and gari fed albino rats compared with their controls. There was no significant difference in the enzymes activities between the sugar and gari fed rats .The study showed that 20%sugar and 20% gari reduced enzyme induction caused in rats fed crude oil contaminated diet through reducing cAMP concentrations. The reduction was not significant between the sugar and gari fed . Therefore sugar and gari diets can repress enzymes induction through the process of glucose effect.

Key words: Glucose Effect % Sugar % Gari % Crude Oil

INTRODUCTION

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 [1]. In several organs, mainly heart and liver, cell damage is followed by increased levels of a number of cytoplasmic enzymes in the blood, a phenomenon that provides the basis for clinical diagnosis of heart and liver diseases e.g. liver enzymes are usually raised in acute hepatotoxicity but tend to decrease with prolonged intoxication due to damage to the liver cells.

Feeding varieties of carbohydrates (Glucose, sucrose and fructose) to mammals and bacteria result in blocking the induction of many enzyme systems. Melvin and Goldberg [2] showed that glucose feeding causes in both man and microorganism profound changes in metabolism include inhibition of induction of several enzymes, stimulation of others and blockage of most effects of glucocorticods while Bostford and Harman [3] studied the reduction of gene expression by glucose (catabolite repression) in various microorganisms. The sugar cane is one of the most important sucrose sources, containing until 20% wt sucrose [4]. The sucrose is a natural sweetener, traditionally, used in human nourishment due to its pleasant taste, nutritious value and low cost production. Sucrose hydrolysis produces a fructose and glucose equimolar mixture named inverted sugar, which has higher edulcorant power.

Cassava is a staple food in human diets in over 80 countries [5]. Gari a starchy food prepared from cassava (*Manihot utilisima*) tubers is one of the most popular

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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. An overall reduction in the activity of the succinate dehydrogenase and cytochrome c oxidase has been reported in albino mice fed on maize (control) and gari (a dried cassava product) based diets for 5 weeks [6] while Chilaka *et al*. [7] reported changes in the activity rates of glucose-6-phosphatase, NADPH-cytochrome c ($P\text{-}_{450}$) reductase, NADPH- dichlorophenol indophenol reductase, cytochrome P_{450} peroxidase and aniline hydroxylase and glucose-6- phosphatase in rats fed Gari (56%w/w) for 9 weeks.

The aim of this study was to compare the effects of sugar and gari diets on hepatotoxic effects caused by crude oil in albino rats using aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alkphos) and Gamma glutamyl transpeptidase (GGT) 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 14days prior to commencement of study. The granulated sugar (produced by Dangote Sugar Nigeria PLC) used in this study was purchased from Mile 3 Market, Port Harcourt.

Animal Studies: Preliminary study was done by the authors to ascertain the oral LD_{100}, LD_{50} and sugar and gari concentrations that will cause glucose effect in rats. Concentrations of 3.88, 7.75, 15.51, 31.01 and 62.02g/kg were the crude oil concentration used as these will be tolerated throughout the study without causing death to the animals(the highest being one half of the LD_{50}). Six groups (in a treatment) of animals were fed adlibitum with crude oil contaminated diets (at concentrations of 3.88, 7.75, 15.51, 31.01, 62.02g/kg) mixed with 20% sugar or 20% gari respectively.

Biochemical Studies: Determination of ALT and AST was done by monitoring the concentrations of pyruvate hydrazone formed with 2, 4 dinitrophenylhydrazine. 0.5ml of buffer solution was dispensed into test tubes labeled blank, sample, control blank and control respectively for AST and ALT, respectively. 0.1ml of sample and control was dispensed into their respective test tubes. All the tubes were incubated at 37°C for 30minutes. 0.5ml of 2, 4 dinitrophenylhydrazine was dispensed into all test tubes. 0.1ml of sample and control was dispensed into their respective blank test tube. The contents of each test tube was mixed and allowed to stand for 20minutes at 25°C. 5ml of 0.4N sodium hydroxide was added to each tube, mixed and read at 550nm against the respective blank prepared. The activity of the unknown was extrapolated from the calibration curve already prepared [8].

Alkaline Phosphatase activity was done by Phenolphthalein Monophosphate method .The test tubes were respectively labeled sample, standard and control. 1.0ml of distilled water was pipetted into each tube followed by a drop of the substrate into each test tube. All the test tubes were incubated at 37°C for 5minutes. 0.1ml of sample, standard and control were dispensed into their respective test tubes. The test tubes were incubated at 37°C for 20minutes. 5ml of colour developer was added to each test tube, mixed and read at 550nm using water as blank. The activity of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard [9].

Gamma Glutamyl Transpeptidase was done by Modified Szasz method [10]. 2.0ml of working reagent (Substrate dissolved in Buffer according to manufacturer's specification) was pipetted into test tube and incubated at 37°C for 3minutes. 0.2ml of serum sample was added into the test tube mixed and transferred into measuring cuvette. The absorbances were read at O, 1, 2 and 3 minutes using water as blank at wavelength of 405nm. The activity of Gamma glutamyltranspeptidase was calculated by multiplying mean change in absorbance per minute with a factor (1158).

Statistical Analysis: The biochemical data were subjected to some statistical analysis. Values were reported as Mean ± 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

The alkaline phosphatase activity of sugar treated albino rats of the control was 41.20 ± 4.42. At 3.88g/kg of sugar treatment, the alkaline phosphatase activity was 27.20 ± 3.93, while it increased to 39.60 ± 2.50, 41.80 ± 3.86,

43.00±2.87 and 43.20±6.15 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg, respectively. The alkaline phosphatase activity of control in gari treated albino rats was 41.60 ±4.27. At 3.88g/kg of gari treatment, the alkaline phosphatase activity was 28.20±8.58, while it increased to 32.80±5.23, 43.40±5.07, 40.60±6.38 and 44.80±3.43 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg, respectively.

The Aspartate amino transferase activity of 14.80 ±3.40 was obtained in the control of gari treated albino rats which increased to 16.40± 4.61 at 3.88g/kg. The Aspartate amino transferase activity further increased to 18.80±3.01, 21.60±3.01, 26.00±5.62 and 28.20±4.19 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively.The Aspartate amino transferase activity of 14.20±2.73 was obtained in the control of sugar treated albino rats which increased to 13.40± 3.64 at 3.88g/kg. The Aspartate amino transferase activity further increased to 17.60 ± 3.53, 20.80 ± 6.29, 24.60 ± 8.90 and 25.60 ± 7.31 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg, respectively.

The sugar treated albino rats also had dose dependent concentration of alanine amino transferase activity (U/L) lower than the petroleum treated albino rats. The alanine amino transferase activity of 11.80 ± 1.93 was obtained in the control of sugar treated albino rats which decreased to 9.80±1.28 at 3.88g/kg. The alanine amino transferase activity further increased to 10.00 ± 2.17, 11.80 ± 1.43,18.40 ± 4.41 and 20.40 ± 6.32 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively. The alanine amino transferase activity of 11.40 ± 1.50 was obtained in the control of gari treated albino rats which reduced to 10.80±2.82 at 3.88g/kg. The alanine amino transferase activity further increased to 12.20 ± 2.87, 12.90 ± 2.08, 16.80 ± 5.57 and 17.80 ± 2.61 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown in Table 1.

The gammaglutamyl transpeptidase activity of control in sugar treated albino rats was 577.00 ± 55.87. At 3.88g/kg of sugar treatment, the gammaglutamyl transpeptidase activity was 315.80 ± 93.80, while it increased to 425.40 ± 74.28, 459.80 ± 76.38, 573.20 ±81.58 and 682.20 ± 43.63 at concentrations of 7.75,15.51, 31.01 and 62.02g/kg respectively. The gammaglutamyl transpeptidase activity of control in gari treated albino rats was 530.80 ± 50.36. At 3.88g/kg of gari treatment, the gammaglutamyl transpeptidase activity was 296.20±92.21, while it increased to 424.60 ± 53.67, 422.00 ± 110.56,

Table 1: Effect of sugar on alkaline phosphatase, aspartate amino transferase and alanine amino transferase in albino rats treated with petroleum

Concentration	Alkaline Phosphatase(u/l)			Aspartate Amino Transferase (U/l)			Alanine Amino Transferase (U/l)		
(g/Kg)	Sugar Treated	Gari Treated	P Value	Sugar Treated	Gari Treated	P Value	Sugar Treated	Gari Treated	P Value
0.00	41.20 ± 4.42	41.60 ± 4.27	0.959	14.20 ± 2.73	14.80 ±3.40	0.916	11.80 ± 1.93	11.40 ± 1.50	0.541
3.88	27.20 ± 3.93	28.20 ± 8.58	0.916	13.40 ± 3.64	16.40 ±4.61	0.565	9.80 ± 1.28	10.80 ± 2.82	0.737
7.75	39.60 ± 2.50	32.80 ± 5.23	0.188	17.60 ±3.64	18.80 ± 3.01	0.728	10.00 ± 2.17	12.20 ± 2.87	0.421
15.51	41.80 ± 3.86	43.40 ± 5.07	0.862	20.80 ± 6.29	21.60 ±4.34	0.905	11.80 ± 1.43	12.80 ± 2.08	0.662
31.01	43.00 ±12.87	40.60 ± 6.38	0.892	24.60 ± 8.90	26.00 ± 5.62	0.856	18.40 ±4.41	16.80 ± 5.57	0.852
62.02	43.20 ± 6.15	44.80 ± 3.43	0.760	25.60 ± 7.31	28.20 ± 4.18	0.660	20.40 ± 6.33	17.80 ± 2.61	0.638

Table 2: Effect of sugar on gamma glutamyl transpeptidase in albino rats treated with petroleum

Concentration (G/kg)	Sugar Treated (U/l)	Gari Treated (U/l)	T	P Value
0.00	577.00 ± 55.87	530.80 ± 50.36	0.181	1.619
3.88	315.80 ± 930.8	296.20 ± 92.21	0.906	0.126
7.75	425.40 ± 74.28	424.60 ± 54.67	0.995	0.700
15.51	459.80 ±76.38	422.00 ± 110.56	0.785	0.291
31.01	573.20 ± 81.58	718.00 ± 65.30	-1.656	0.173
62.02	682.20 ± 43.63	750.40 ± 130.57	-0.450	0.678

Table 3: Effect of sugar and gari on hepatic enzymes in albino rats treated with petroleum

Parameter	Sugar	Gari	P Value	Control	Sugar	P Value	Control	Gari	P Value
Alkphos (U/L)	38.96±3.00	37.96±3.20	0.829	47.20± 7.37	38.96±3.00	0.457	47.20± 7.37	37.96±3.20	0.408
ALT (U/L)	14.08±2.22	14.08±1.36	0.967	10.80± 2.04	14.08±2.22	0.443	10.80± 2.04	14.08±1.36	0.361
AST(U/L)	20.40±2.26	22.20±2.19	0.444	18.00±2.95	20.40±2.26	0.632	18.00±2.95	22.20±2.19	0.342
GGT (U/L)	491.28± 62.96	522.24±89.74	0.700	513.00±56.60	491.28±62.96	0.841	513.00±56.60	522.24±89.74	0.942

718.00±65.30 and 750.40 ± 130.57 at concentrations of 7.75, 15.51, 31.01 and 62.02g/kg respectively as shown in Table 2.

Overall, there was no significant difference in 38.96 ± 3.00 alkaline phosphatase (U/L) activity of sugar fed rats compared with 38.57 ± 2.40 of gari fed rats. Also ALT (U/L) activity of 14.08 ± 2.22 in sugar treated rats was not significantly different from 13.63 ± 1.28 in gari treated rats. The AST (U/L) activity of 20.40 ± 2.26 in sugar treated rats was not significantly different from 20.97 ± 1.83 in gari treated albino rats. The GGT activity (U/L) of 491.28 ± 62.94 in petroleum treated rats was not significantly different from 523.67 ± 44.94 in gari treated rats as shown in Table 3.

DISCUSSION

There were dose dependent increases in sugar and gari fed rats treated with crude petroleum compared with their controls. This is similar to study of Michaelis and Szepesi [11] which reported dose dependent increase in hepatic glucose 6 phosphate dehydrogenase (G6PD), Malic enzyme and relative liver weight when rats were fed various concentrations of glucose, sucrose, galactose, fructose and lactose with 10% of glucose, fructose and sucrose showing the least enzymes induction. This suggests that both glucose and gari causes repression of enzymes induction. Braide *et al.* [12] reported that gari and sugar caused glucose effect at 20% concentration by lowering enzymes activity at this concentration through reducing cAMP concentration.

There was no significant difference in the alkaline phosphatase, aspartate amino transferase, aspartate amino transferase and gamma glutamyl transpeptidase activities of the sugar and gari treated albino rats on crude petroleum toxicity. This may be as a result of sugar and gari being a carbohydrate whose end product include glucose. Glucose represses the induction of inducible operons by inhibiting the synthesis of cyclic Adenosine monophosphate (cAMP). In the absence of glucose, cAMP levels are high, CAP is activated by cAMP and transcription occurs (in the presence of lactose). Thus the effect of sugar and gari in suppressing these inducible enzymes is by lowering cyclic AMP level. Braide *et al.* [13] reported that gari diet reduced enzymes activities in rats fed crude oil contaminated diets while feeding on sugar diet has been shown to reduce haematoxicity caused by petroleum in albino rats [14].

The rats fed sugar had insignificant lower enzymes concentration than the gari fed albino rats. This is similar to result by Ezeji *et al.* [6] using maize and gari. Ezeji *et al.* [6] had suggested that cyanide affects some important enzymes of the mitochondrial electron transport system. The presence of cyanogens in the gari caused the slight increases in enzymes activities in gari fed rats compared with sugar fed albino rats. Carbohydrate administration reportedly decreased the level of the mitochondrial enzyme ä- aminolaevulinic acid synthetase in liver [15] and hepatic dimethylaminoazobenzene reductase in a similar manner [16] while an elevated level of hepatic phosphoenolpyruvate carboxykinase was suppressed by administration of glucose or glycerol [17, 18]. Thus the insignificant difference observed in the overall parameters may be as result of the fact that gari and sugar are both carbohydrate whose end product is glucose thus the same effect on crude oil toxicity.

CONCLUSION

This study has shown that feeding on granulated sugar and gari represses the induction of enzymes. The study also revealed that there was no difference in the repression effect of both gari and sugar. These may be attributed to the fact that the breakdown of both granulated sugar and gari yielded glucose ultimately causing glucose effect.

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