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Impact of Prolonged Intake of Sugar-Free Carbonated Soft Drink on Selected Biochemical Indices of Male Wistar Rats

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Abstract

The impact of prolonged intake of sugar-free carbonated soft drinks on selected biochemical indices of adult male Wistar rats were studied. Thirty (30) wistar rats weighing between 175g and 225g were used in this study. They were separated into five (5) groups of six (6) rats each. Animals in groups 1, 2, 3, 4, and 5 were fed normal feed and water *ad libitum* and in addition, groups 2, 3, 4 and 5 received 1.4mlkg^{-1} B.W of popular regular cola drink, 3mlkg^{-1} B.W of popular regular cola drink, 1.4mlkg^{-1} B.W of sugar-free cola brand and 3mlkg^{-1} B.W of sugar-free cola brand respectively. Three experimental animals were sacrificed after 3 and 6 weeks of treatment and selected biochemical indices were assayed. Biochemical assessment showed significant increase ($p \leq 0.05$) in Alanine aminotransferase (ALT) activities across the group for 3 and 6 weeks respectively, while Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities across the group for 3 weeks decreased, and were later observed to increase in the group administered 3mlkg^{-1} B.W of popular regular cola drink and sugar-free cola brand for 6 weeks at tested concentrations and duration when compared with control. Results from urea concentration was observed to increase significantly ($p \leq 0.05$) in groups administered popular regular cola drink and sugar-free cola brand for 3 weeks and 6 weeks respectively, when compared with the control. Also, there was an increase in creatinine concentration with the administration of popular regular cola and sugar-free cola brand across the group for 3 and 6 weeks. There were no significant changes in Sodium ion (Na^+) and Potassium ion (K^+) in all the groups administered popular regular cola drink and sugar-free brand. However, differences observed in Triglyceride (TG), Total Cholesterol (TC), High Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) across all the groups for 3 weeks and 6 weeks showed that TG, TC and HDL decreased in comparison with the control, while in LDL, there was significant increase. The study suggests that regular cola and sugar-free cola may be predisposing factors for cardiovascular diseases as observed in elevated LDL levels. Generally, extension of the treatment for a long time may actually predispose the animals to nephrotic and cardiovascular diseases.

Introduction

Sugar-free products are sweetened by sugar substitutes known as artificial sweeteners that produce a low glycemic response and contain few to no calories. They are widely used in processed foods such as baked goods, powdered drink, mixes, puddings, jams, etc. (Findikli and Turkoglu, 2014). Non- nutritive sweeteners have been subjected to intense scrutiny and critics maintain that sweeteners induce a variety of unhealthy

conditions including cancer (Findikli and Turkoglu, 2014). Some studies have associated artificial sweetener with bladder cancer in laboratory rats, and high sugar consumption is linked with dental caries, obesity, cardiovascular diseases and diabetes . The United States of America have ensured that food containing saccharin is labeled with warning that the use of the product could be hazardous to health (Williams, 2002). Similarly, the increased prevalence of obesity in children is a major challenge coincided with astronomical increase in the consumption of sugar-sweetened beverages (Duffey and Popkin, 2007). These beverages are considered more fattening than solid foods and thus, children who increase their consumption of sugar sweetened beverages may not reduce intake of calories from other foods and beverages with a resultant increase in total energy and weight gain (Findikli and Turkoglu, 2014). The exact composition of sweetened beverages is confidential but the main known components include phosphoric acid, water, sugar (sucrose) and caffeine (Tothova *et al*., 2013). A correlation exists between soft drinks consumption and the incidence of multiple diseases such as diabetes, kidney stone formation, bone problems and cardiovascular diseases (Fung *et al*., 2009). Sugar is substituted by artificial sweeteners in sweetened beverages, and studies have shown the deleterious consequences some of the contents of these soft drinks exert on human health (Alkhedaide *et al*., 2016). Soft drink consumption may cause bone fracture, disruption in bone formation, urinary calcium depletion, diabetes and cardiovascular diseases . Only few studies have examined the possible harmful effects of sweetened beverages on the nephron and hepatocytes. Consequently, there is need to provide proper evidence on the impact of prolonged intake of popular regular cola and sugar-free cola drinks on the liver markers, lipid profile and renal markers in male mammals. Hence, the study is aimed at highlighting the impact of prolonged intake of popular regular cola and sugar-free cola drinks on selected biochemical indices in male Wistar rats**.**

Materials and Methods

Carbonated Soft Drinks

Carbonated soft drinks (popular regular and sugar-free cola drinks) used in this study were purchased from a soft drink vendor in Port Harcourt, Nigeria in July, 2017 and stored under standard laboratory conditions.

Study Participants

Thirty (30) adult male Wistar rats weighing between175g and 225g were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt. They were housed in cages with different compartments and fed *ad libitum* with water and Top feeds and were acclimatized for a week under standard laboratory conditions of relative humidity , adequate ventilation and ambient room temperature . The experimental rats were weighed and reweighed after acclimatization and assigned into five (5) study groups of six (6) rats per group and their weights after a week of acclimatization served as the initial weight for the experimental studies.

Experimental Design

Treatments commenced after acclimatization and the rats were treated for 3 weeks and six weeks with popular regular cola and sugar-free cola drinks. Group 1 rats received oral dose of normal feed and water *ad libitum* for three weeks and six weeks and served as the control. Group 2 rats received oral dose of popular regular cola 1.4mlkg^{-1} B.W for three weeks and six weeks, Group 3 rats received oral dose of popular regular cola 3mlkg^{-1} B.W for three weeks and six weeks, Group 4 rats received oral dose of sugar-free cola brand 1.4mlkg^{-1} B.W for three weeks and six weeks while Group five rats received oral dose of sugar-free cola drinks 3mlkg^{-1} B.W for three weeks and six weeks.. Organization of Economic Corporation and Development (OECD) guidelines on direct calculation of animal dose from human dose was adopted for estimation of dose administered (OECD, 2000). The rats were sacrificed by cervical dislocation and blood samples were obtained after three weeks and six weeks via cardiac puncture and dispense into the Heparin bottle for biochemical assay and determination of livermarkers (AST, ALP and ALT), renal markers (Urea, Creatinine, N^+ and K^+) and lipid profile (HDL-cholesterol, TG, TC and LDL-cholesterol) in the experimental rats .

Statistical Analysis of Data

All data were subjected to statistical analysis using statistical package for social science. SPSS software version 20. Mean values (M) ± SEM were calculated and One-Way ANOVA (Analysis of Variance) test was performed. Significance level was considered at 95 % confidence level ($p \leq 0.05$)

Biochemical Assay

Estimation of creatinine concentration was analyzed with Kinetic Methods Kits from Randox (United Kingdom) using a double beam spectrophotometer. Estimation of urea concentration was carried out using Randox Laboratories Kits (United Kingdom). Sodium and potassium ions electrolytes were analysed and estimated using EUC machine. Estimation of alkaline phosphatase was carried out using QCA Kits Method. Estimation of alanine aminotransferase was determined using Randox Laboratories Kits Method. Determination of aspartate aminotransferase was estimated by using Randox Laboratories Kits. Plasma cholesterol, HDL-cholesterol and triglyceride were determined and estimated using Randox Laboratories Kits Method.

Results

Liver enzyme markers of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3 weeks

The results for the studied liver enzyme markers of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola brands for 3 weeks are presented in Table 1. Results showed significant ($p \leq 0.05$) decreases in AST and ALP concentrations in all but group 3 when compared with the control, while a major significant ($p \leq 0.05$) increase was detected in all four (4) groups when compared with control in ALT concentrations for the group administered both 1.4mlkg^{-1}and 3mlkg^{-1}of popular regular cola and sugar-free cola drinks

Table 1. Liver enzyme markers of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3weeks

Groups	ALT (µl^{-1})	AST (µl^{-1})	ALP (µl^{-1})
Control	17.33±5.20 a	70.66±12.41^{a}	73.46±9.07^{a}
Normal cola 1.4mlkg^{-1}	24.00±2.51 ^{b}	52.66±3.48 ^{b}	52.66±5.45^{b}
Normal cola 3mlkg^{-1}	29.00±2.08 ^{b}	71..33±6.38^{a}	72.66±8.66 ^{a}
Sugar free cola1.4mlkg^{1}	22.33±1.45^{b}	41.33±3.17^{b}	44.00±4.16^{b}
Sugar free cola 3mlkg^{-1}	30.00±1.15^{b}	44.33±4.25^{b}	46.00±10.58^{b}

Data are mean values ± SEM, n=3. Values in the same column and bearing same superscript letters with control are not significant ($p \geq 0.05$) compared to control, whereas values in the same column and bearing different superscript letters from control are considered significant ($p \leq 0.05$) in relation to the control.

Liver enzyme markers of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 6 weeks

The results of liver enzyme markers of male Wistar rats administered different concentrations of popular regular cola and sugar free cola brands for 6 weeks are presented in Table 2. Results showed significant ($p \leq 0.05$) increase in AST and ALP concentrations across the groups when $3mlkg^{-1}$ of both sugar-free cola drinks and regular cola were administered, and a considerable significant ($p \leq 0.05$) increase was also observed in ALT concentrations across all four (4)groups administered $1.4mlkg^{-1}$ and $3mlkg^{-1}$of popular regular cola and sugar-free cola drinks compared to the control.

Table 2. Liver enzyme markers of male Wistar rats administered different concentrations of popular regular cola and sugar free cola drinks for 6weeks

Groups	ALT (μl^{-1})	AST (μl^{-1})	ALP (μl^{-1})
Control	16.66 ± 5.81^a	70.66 ± 12.41^a	73.46 ± 9.07^a
Regular cola $1.4mlkg^{-1}$	24.33 ± 4.63^b	69.33 ± 3.38^a	65.56 ± 6.61^b
Regular cola $3mlkg^{-1}$	25.00 ± 6.65^b	82.00 ± 3.78^b	75.96 ± 1.85^a
Sugar free cola$1.4mlkg^{1}$	25.00 ± 3.46^b	65.00 ± 7.00^a	66.46 ± 12.43^a
Sugar free cola $3mlkg^{-1}$	35.66 ± 1.66^b	75.33 ± 6.76^b	82.53 ± 6.76^b

Data are mean values ± SEM, n=3. Values in the same column and bearing same superscript letters with control are not significant ($p \geq 0.05$) compared to control, whereas values in the same column and bearing different superscript letters from control are significant ($p \leq 0.05$) compared to control.

Renal function indices of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3 weeks

The outcome of the renal function indices of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3 weeks are presented in Table 3. The results for plasma electrolytes showed no significant change ($p \geq 0.05$) in Sodium ion (Na^+) and Potassium ion (K^+) concentrations across all groups while a significant ($p \leq 0.05$) increase was observed in urea concentrations in the groups administered $1.4mlkg^{-1}$and $3mlkg^{-1}$of popular regular cola and $3mlkg^{-1}$ of sugar-free cola drinks in comparison with the control group. In addition, a significant ($p \leq 0.05$) increase was observed in creatinine concentrations in groups administered $3mlkg^{-1}$ of popular regular cola and $3mlkg^{-1}$ of sugar-free cola drinks when compared with the control group.

Table 3. Plasma renal indices of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3 weeks

Groups	Urea**(mmoll^{-1})**	Creatinine**(µmoll^{-1})**	Na^+**(mmoll^{-1})**	K^+**(mmoll^{-1})**
Control	6.96±0.54^a	48.00±0.54^a	134.00±0.57a	4.16±0.18^a
Regular cola 1.4mlkg^{-1}	9.10±0.72^b	52.33±4.30^a	135.00±0.57^a	4.43±0.14^a
Regular cola 3mlkg^{-1}	11.03±0.44^b	57.06±3.88^b	133.66±0.33^a	4.26±0.17^a
Sugar free cola1.4mlkg^1	7.86±0.91^a	52.83±3.96^a	136.66±3.47^a	4.76±0.26^a
Sugar free cola 3mlkg^{-1}	9.03±0.20^b	56.53±3.47^b	134.33±0.66^a	4.93±0.06^a

Data are mean values ± SEM, n=3. Values in the same column and bearing same superscript letters with control are not significant ($p \geq 0.05$) in comparison with the control whereas values in the same column and bearing different superscript letters from control are significant ($p \leq 0.05$) compared to the control.

Renal function indices of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 6 weeks

The outcome of the renal function indices of male Wistar rats administered different concentrations of popular regular cola and sugar free cola drinks for 6 weeks are presented in Table 4. The results for the electrolyte levels showed no significant changes ($p \geq 0.05$) in Sodium ion (Na^+) and Potassium ion(K^+) concentrations across all groups while a significant ($p \leq 0.05$) increase was observed in urea concentrations in groups administered 1.4mlkg^{-1} and 3mlkg^{-1} of regular cola and 3mlkg^{-1} of sugar-free cola drinks in comparison with the control group. In addition, a significant ($p \leq 0.05$) increase was observed in creatinine concentrations across all groups administered popular regular cola and sugar-free cola drinks when compared with the control group.

Table 4. Renal function indices of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 6 weeks

Groups	Urea**(mmoll^{-1})**	Creatinine**(µmoll^{-1})**	Na^+**(mmoll^{-1})**	K^+**(mmoll^{-1})**
Control	7.00±0.57^a	46.00±0.96^a	134.00±0.96^a	4.16±0.18^a
Regular cola 1.4mlkg^{-1}	9.00±0.54^b	65.66±3.17^b	135.00±0.57^a	4.43±0.14^a
Regular cola 3mlkg^{-1}	13.66±0.38^b	57.66±3.84^b	134.00±0.57^a	4.26±0.17^a
Sugar-free cola1.4mlkg^1	7.76±0.31^a	63.40±1.08^b	136.66±0.33^a	4.76±0.26^a
Sugar free cola 3mlkg^{-1}	11.20±0.26^b	51.30±2.89^b	136.00±0.57^a	4.93±0.06^a

Data are mean values ± SEM, n=3. Values in the same column and bearing same superscript letters with control are not significant ($p \geq 0.05$) compared to the control whereas values in the same column and bearing different superscript letters from control are significant ($p \leq 0.05$) compared to the control.

Plasma lipid profile of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3 weeks

Plasma lipid profile of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3 weeks are presented in Table 5. Results showed no significant ($p \geq 0.05$) differences in Triglyceride (TG), Total Cholesterol (TC) and High-Density Lipoprotein (HDL) levels across all treated groups in comparison with the control group, while in Low Density Lipoprotein (LDL), there was considerable elevation across all except group 2 treated with both drinks.

Table 5. Plasma lipid profile of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3 weeks

Groups	TC **($mmoll^{-1}$)**	TG **($mmoll^{-1}$)**	HDL-Cholesterol **($mmoll^{-1}$)**	LDL-Cholesterol **($mmoll^{-1}$)**
Control	3.10 ± 0.49^{a}	0.96 ± 0.17^{a}	0.93 ± 0.06^{a}	2.06±0.01a
Regular cola 1.4$mlkg^{-1}$	2.70 ± 0.40^{a}	1.10 ± 0.10^{a}	0.63 ± 0.08^{a}	2.53 ± 0.33^{a}
Regular cola 3$mlkg^{-1}$	2.43 ± 0.44^{a}	0.70 ± 0.05^{a}	0.63 ± 0.08^{a}	4.46 ± 0.32^{b}
Sugar free cola1.4$mlkg^{1}$	2.33 ± 0.31^{a}	0.73 ± 0.06^{a}	0.60 ± 0.05^{a}	3.06 ± 0.06^{b}
Sugar free cola 3$mlkg^{-1}$	2.06 ± 0.17^{a}	0.80 ± 0.17^{a}	0.63 ± 0.12^{a}	4.36 ± 0.23^{b}

Data are mean values ± SEM, n=3. Values in the same column and bearing same superscript letters with control are not significant ($p \geq 0.05$) in relation to the control, while values in the same column bearing different superscript letters from control are significant ($p \leq 0.05$) compared to the control.

Plasma lipid profile of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 6 weeks

Plasma lipid profile of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 6 weeks are presented in Table 6. Results showed no significant ($p \geq 0.05$) differences in Triglyceride (TG), Total cholesterol (TC) and High density lipoprotein (HDL) levels across all treated groups in comparison with the control group. However, a significant ($p \leq 0.05$) increase was observed in LDL levels in group administered 1.4$mlkg^{-1}$and 3$mlkg^{-1}$ of sugar-free cola drink and 3$mlkg^{-1}$ of regular cola when compared with those of the control group.

Table 6. Plasma lipid profile of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 6 weeks

Groups	TC **($mmoll^{-1}$)**	TG **($mmoll^{-1}$)**	HDL-Cholesterol **($mmoll^{-1}$)**	LDL-Cholesterol **($mmoll^{-1}$)**
Control	2.86 ± 0.33^{a}	1.06 ± 0.18^{a}	0.93 ± 0.06^{a}	2.00 ± 0.15^{a}
Regular 1.4$mlkg^{-1}$	3.33 ± 0.38^{a}	0.70 ± 0.05^{a}	0.63 ± 0.08^{a}	2.66 ± 0.17^{a}
Regular 3$mlkg^{-1}$	2.96 ± 0.34^{a}	1.10 ± 0.05^{a}	0.63 ± 0.08^{a}	5.16 ± 0.12^{b}
Sugar free 1.4$mlkg^{1}$	2.10 ± 0.10^{a}	1.36 ± 0.20^{a}	0.60 ± 0.05^{a}	3.76 ± 0.03^{b}
Sugar free 3$mlkg^{-1}$	2.50 ± 0.40^{a}	0.76 ± 0.14^{a}	0.63 ± 0.12^{a}	4.23 ± 0.32^{b}

Data are mean values ± SEM, n=3. Values in the same column and bearing same superscript letters with control are not significant ($p \geq 0.05$) compared to the control whereas values in the same column and bearing different superscript letters from control are significant ($p \leq 0.05$) compared to control.

Discussion

Cola drinks contain many different chemical compounds and no certainty exists as to which ones may be associated with disease risk (Imai *et al*., 2010). Epidemiological studies have shown that consumption of sugar or sugar-sweetened beverages is associated with unfavourable lipid concentrations, insulin resistance, fatty liver disease, type-2 diabetes, cardiovascular disease and metabolic syndrome (Yoshida *et al*., 2007). More often than not, hepatotoxic agents increase the release of certain enzymes such as aspartate aminotransferase and alanine aminotransferase into the bloodstream as a result of damage to liver cells (Ben-Chioma *et al*., 2015). The present study demonstrated a significant increase in ALT activity across all the group administered 1.4$mlkg^{-1}$ and 3$mlkg^{-1}$of popular regular cola and sugar-free cola brands (Table 1 and 2) and a significant increase in AST and ALP activities across the groups for six weeks of treatment when compared with the control group. The report of the current study is in consonance with the submission of Ben-Chioma *et al*., (2015), that reported increase in AST, ALP and ALT activities showed by both coke products reflects mild toxic effects on the liver. Aspartate aminotransferases are present in high concentrations in a number of tissues such as liver, kidney, heart and pancreas, and are released slowly in comparison to ALT (Ben-Chioma *et al*., 2015). However, ALT is localized primarily in the cytosol of hepatocyte. This enzyme is considered a more sensitive marker of hepatocellular damage than AST (Al-Mamary *et al*., 2002). The elevations in the activities of ALT, AST and ALP in the present study suggests mild toxicity which permitted leakage of the enzyme

from the hepatocytes into the bloodstream and this outcome could be attributed to the high caloric value and added sugars such as sucrose or high fructose corn syrup, aspartame and Caramel colourant present in popular regular cola and sugar-free cola brands (Vlassara *et al*., 2002). The observation in relation to the elevated concentrations of ALT in the current study corroborates the findings of Alkhedaide *et al*. (2016) who observed elevated concentrations of ALT in male Wistar rats treated with different brands of soft drinks. The present study reported significant ($p \leq 0.05$) increases in urea and creatinine levels in male Wistar rats treated with popular regular cola and sugar-free cola brands (Table 3 and 4). The results of this study complement a growing body of literature tying sugar-sweetened soda consumption to hyperuricemia and renal injury (Shoham *et al.,* 2008). The observed significant increase in urea and creatinine concentrations in groups administered popular regular cola and sugar-free cola brands in this study suggests that these carbonated drinks (regular cola and sugar-free cola) may have acted as toxins to the nephrons resulting in distortion, disruption or possible impairment in renal function. These observations are in consonance with the reports of Adjene *et al*. (2010). They implicated soda pop drinks as capable of precipitating kidney disease probably by causing congestion and tubular necrosis of the kidney. The results of the lipid profile analysis in this study (Tables 5 and 6) demonstrated decreased levels of TC, TG and HDL-Cholesterol though not significant ($p \leq 0.05$) in male Wistar rats treated with sugar-free cola and regular cola, while LDL shows a significant ($p \leq 0.05$) increase. Epidemiological studies have shown that consumption of sugar or sugar-sweetened beverages is associated with unfavourable lipid concentrations, insulin resistance, fatty liver disease, type-2 diabetes, cardiovascular disease and metabolic syndrome (Yoshida *et al*., 2007). The popular regular cola and sugar-free cola drinks exhibited decreased levels of TC, TG and HDL-cholesterol though not significant; this could be attributed to the presence of added sugars and aspartame content in sugar-free cola and regular cola. Aspartame is used in sugar-free cola and is metabolized in the gut to phenylalanine, methanol and aspartic acid. After chronic intake of high levels of aspartame, the produced methanol, as well as its metabolites, formaldehyde and formic acid, increase oxidative and carbonyl stress in the rat brain (Iyyaswamy and Rathinasamy, 2012).

Conclusion.

The findings of this study have shown that popular regular and sugar-free cola drinks are likely to predispose one to biochemical imbalance. Impact of prolonged intake of regular cola and sugar-free cola drinks were observed to increase the concentrations of ALT, AST, ALP, Urea and Creatinine which are markers for hepatic and nephrotic status. The observed increases in LDL levels across all groups suggest that regular cola and sugar-free cola drinks may be predisposing factors for cardiovascular diseases.

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