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Impact Of Prolonged Intake Of Sugar-Free Carbonated Soft Drinks On Reproductive Outcomes Of Male Wistar Rats.

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Abstract: *The impacts of prolonged intake of sugar-free carbonated soft drink on reproductive parameters of adult male Wistar rats were studied. Thirty (30) wistar rats weighing between 175g to 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, 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 reproductive parameters were assayed. Reproductive assessment showed reduction in testosterone levels with administration of both cola drinks though not significant ($p \geq 0.05$). Administration of the popular regular cola and sugar free cola drink raised observed sperm morphology and percentage death. However, there was an observed decrease in sperm motility, and count in sugar-free cola. The study suggests that prolonged administration of regular cola and sugar-free cola may be predisposing factors for abnormal disturbance in sperm quality parameter and testosterone level and is a possible indication of fertility problem. Generally, extension of the treatment for a long period may actually predispose the animals to fertility problems.*

Key Words: Sugar-free drinks, Sperm quality, Testosterone.

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I. 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 drinks, 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 sweeteners 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 male reproductive organs. Consequently, there is need to provide proper evidence on the sperm quality and testosterone levels 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 the sperm quality and testosterone levels in male Wistar rats.

II. Materials and methods

2.1 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.

2.2 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 weight after a week of acclimatization served as the initial weight for the experimental studies.

2.3 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 were adopted for estimation of dose administered (OECD, 2000). The rats were sacrificed by cervical dislocation and dissected. Blood samples and semen from epididymis of the testes were obtained after three weeks and six weeks of treatment. Blood collected via cardiac puncture was dispensed into Lithium heparin bottle for hormonal assay, while the semen was dispensed into universal container for semen analysis of the experimental rats.

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2.4 Statistical analysis of data

Statistical analysis of data was done 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 ($p \leq 0.05$)

2.5 Biochemical assay

Estimation of testosterone level of the experimental rats was carried out using the method employed by microplate automated reader using Clinotech enzyme-linked immunosorbent assay (ELISA) hormonal assay kits (Clinotech Diagnostic, Richmond, Canada). This Clinotech enzyme immunoassay test is intended for the quantitative determination of testosterone in serum. The testosterone- enzyme immunoassay (EIA) is based on the principle of competitive binding between testosterone in the test specimen and testosterone conjugate. Semen parameters were determined by using Neubauer Haemocytometre to carry out sperm count under a microscope using × 40 objectives.

. III. Results

Sperm quality analysis of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola brands for 3 weeks

The sperm quality analysis of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola for 3 weeks are presented in Table 1. Result obtained showed significant ($p \leq 0.05$) alterations in sperm morphology and percentage death. There was also an observed decrease in sperm motility and count in sugar-free cola drinks in comparison to the control group.

Table 1. Sperm quality analysis of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 3 weeks

Groups	Abnormal Morphology(%)	Motility(%)	Deadness(%)	Sluggishness(%)	Sperm.Count($x10^6ml^{-1}$)
Control	70.00±2.88[a]	75.00±1.93[a]	20.00±2.88[a]	13.33±1.66[a]	300.00±35.37[a]
Regular 1.4$mlkg^{-1}$	75.00±2.88 [b]	60.00±2.88[b]	30.33±1.66[b]	11.66±1.66 [a]	270.00±14.86[b]
Regular 3$mlkg^{-1}$	80.00±2.87 [b]	61.66±3.33[b]	31.33±2.76[b]	10.33±2.76 [a]	275.33±25.09[b]
Sugar free 1.4$mlkg^{1}$	75.00±2.88[b]	60.00±2.76[b]	40.51±3.33[b]	11.37±0.59 [a]	256.37±22.09[b]
Sugar free 3$mlkg^{-1}$	75.26±5.77[b]	60.20±2.88[b]	25.20±2.88[b]	10.00±0.10[a]	254.00±12.86[b]

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

Sperm quality analysis of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 6 weeks

The sperm quality analysis of male Wistar rats administered different concentrations of popular regular cola and sugar-free cola drinks for 6 weeks are presented in Table 2. Results demonstrated significant ($p \leq 0.05$) changes in sperm morphology and percentage death, while in percentage motility and count, there were a significant ($p \leq 0.05$) decrease in relation with the control.

Table 2. Sperm quality analysis of male Wistar rats administered different concentrations ofpopular regular cola and sugar-free cola drinks for 6 weeks

Groups	Abnormal Morphology(%)	Motility(%)	Deadness (%)	Sluggishness (%)	Sperm.Count ($x10^6ml^{-1}$)
Control	71.20±0.11[a]	73.00±2.88[a]	15.00±2.88[a]	15.00±1.68[a]	283.33±32.64[a]
Regular 1.4$mlkg^{-1}$	76.00±2.88[b]	60.00±5.77[b]	25.33±4.40[b]	6.66±1.40[b]	225.33±16.33[b]
Regular 3$mlkg^{-1}$	80.00±2.88[b]	61.33±5.00[b]	28.00±2.88[b]	11.00±2.08[a]	215.00±35.37[b]
Sugar free 1.4$mlkg^{1}$	75.66±1.66[b]	58.33±4.40[b]	38.50±2.60[b]	11.66±0.66[a]	207.66±.36.60[b]
Sugar free 3$mlkg^{-1}$	81.33±4.40[b]	54.66±1.66[b]	31.33±1.66[b]	10.33±1.66[a]	203.00±25.00[b]

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

Testosterone levels of male Wistar rats administered popular regular cola and sugar-free cola drinks for 3 and 6 weeks

The effect of treatment with sugar-free cola brand and popular regular cola for 3 and 6 weeks on testosterone levels of male Wistar rats are shown in Table 3. Results obtained showed insignificant ($p \geq 0.05$) reduction in testosterone levels across all groups administered various concentrations of regular cola and sugar-freecola drinks for 3 and 6 weeks when compared with the control.

Table 3.Testosterone levels of male Wistar rats administered popular regular cola and sugar- free zero cola brands for 3 and 6 weeks

Testosterone ($ngml^{-1}$)		
Groups	3 weeks	6 weeks
Control	1.23±0.01[a]	1.20±0.11[a]
Regular cola 1.4$mlkg^{-1}$	1.01±0.34 [a]	1.00±0.66[a]
Regular cola 3$mlkg^{-1}$	1.03±0.23 [a]	1.10±0.11[a]
Sugar free cola 1.4$mlkg^{-1}$	1.06±0.35 [a]	0.95±0.10[a]
Sugar free cola 3$mlkg^{-1}$	1.00±0.14[a]	0.91±0.11[a]

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

IV.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). The result of the sperm quality analysis of male Wistar rats (Table 1 and 2) administered regular cola and sugar-free cola drinks in the present study demonstrated significant changes in the analysis of sperm quality parameters. Administration of both cola drinks raised observed abnormal sperm morphology and percentage death and there was an observed decrease in sperm motility and count. Consumption of Sugar sweetened beverages has been found to increase insulin resistance in adolescents (Kondaki et al., 2013) and adults (Stanhope et al., 2009). Insulin resistance is known to increase oxidative stress (Park et al., 2009), which in turn can negatively influence sperm motility (Benedetti et al., 2012; Chen et al., 2013). In addition, conditions characterized by insulin resistance, such as type 2 diabetes, have also been related to lower sperm motility (Echavarria et al., 2007; Rama et al., 2012). A recent Danish study, Jensen et al., (2010),revealed that sperm counts are lower in men who drink 1 Litre of cola estimated to contain (100-140 mg of caffeine) or more per day. This cola effect on sperm seems not to be attributable to their caffeine content alone; caffeine intake of < 800 mg per day is not associated with reduced semen quality (Imai et al., 2010). Aspartame was approved by the Food and Drug Administration (FDA) in 1996 as a general sweetener, since then aspartame is the most commonly used artificial sweetener worldwide (Soffritti et al., 2007). 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). Chronic consumption of cyclamate and acesulfame K, present in sugar-free cola has been shown to lead to testicular abnormalities and carcinomas (Karstadt, 2010; Sasaki et al., 2002). The significant changes observed in sperm quality parameters in male Wistar rats treated with regular cola and sugar-free cola brand in the current study may be attributed to the duration of the study as the study was conducted for 3 and 6 weeks. The results of the testosterone levels of Wistar rats treated with popular regular cola and sugar-free cola drink(Table 3) in the present study showed reduction in testosterone levels though not significant when compared with the control. The report of the current study is consistent with the submissions of Tothova et al., (2013), that reported insignificant differences in plasma testosterone concentrations in rats treated with different sweetened beverages such as regular cola, caffeine free, light and zero cola for six months. The results might be biased by other partially unknown compounds present in the different types of sweetened beverages as documented by Tothova et al., (2013).

V.Conclusion

The consumption of soft drinks has increased considerably during the last decades. Among them, the cola based preparations are possibly the refreshments with the largest sale worldwide (Imai et al., 2010). During the previous years, important concerns have been raised about the effects of colas on human health. In addition to the possible detrimental effects of chronic cola consumption which include enamel softening , bone demineralization, hypokalemic myopathy , development of metabolic syndrome and diabetes mellitus (Imai et al., 2010). However, only few events have validated these claims, therefore the study investigated the impact of prolonged intake of sugar-free carbonated soft drink on reproductive parameters of male Wistar rats. The findings of this study have shown that popular regular and sugar-free cola drinks are likely to predispose one to fertility problems. The observed decrease in gonadal steroid level and abnormal sperm quality indices is an indication of possible fertility issues arising from prolonged intake of such beverage.

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