Original Research Article

REPRODUCTIVE & BIOMARKER RESPONSE OF MALE ALBINO RATS *(Rattus norvegicus)* TO A DAILY DOSE OF SOFT DRINK (COCA-COLA)

Abstract

The effect of Coca-cola was evaluated on Albino rats. The parameters analysed include; Sperm count, kidney function test, liver test, red blood cell, pack cell volume, haemoglobin, white blood cell, platelets, lymphocytes. The results showed that: The mean serum electrolyte for Na was low for week 1, 2, 3 and 4 having 142, 140, 133.6 and 141.66 respectively when compared to the average control (147.3) with a significant difference (P<0.05) in week 1 and 4, K were all lower than the average control (5.4) across the week with no significant difference (P>0.05) but had the least mean value of 4.8 in week 2. Bicarbonate was also significantly lower (P<0.05) in the treated group when compared to the average control (24.3) with the least mean value in week 4 (18.67) and Cl had a mean of 93.0 in week 1, 94.67 in week 2, 108.66 in week 3 and 107.67 in week 4 with an average control of 99.33. AST had a mean of 20.67 in week 1 which increased to 31.67 in week 4 while ALT had a mean of 10 in week 1 which also increased to 13 in week 4. The mean serum protein reduced from 81.83 in week 1 to 73.24 in week 4. Mean PCV reduced from 33.67 in week 1 to 32.7 in week 4, Hb increased from 11.2 in week 1 to 13.4 in week 4 with a significant difference (P<0.05) when comparing the test with the average control, WBC increased from a mean 5.26 in week 1 to 11.9 in week 4 with a significant difference (P<0.05), Platelet had a mean of 315 on week 1 and 419 in week 4 with significant difference (P<0.05) in week 3 and 4 when compared with its control, RBC increased from a mean of 4.23 in week 1 to 6.90 in week 4 with significant difference (P<0.05). Lymphocyte for week 1 had a mean value of 70 in week 1 and 82.26 in week 4 with significant difference (P<0.05) across the week. While the mean sperm count reduced significantly (P<0.05) from 425 in week 1 to 400 in week 4 when compared to the average control (566). These findings demonstrate that regular consumption of Coca-cola had a detrimental effect on the sperm count, liver, kidney and on the haematological parameters.

INTRODUCTION

Coca-cola is one of the world's favorite soft drink, It comprises of kola nut which is a source of caffeine and coca leaves, phosphoric acid, sugar in the form of glucose and other forms of chemical that is used for preservation, flavor and colorings (Adjene *et al.*, 2010). Coca-Cola intake has increased in the past two decades (Nielsen *et al., 2*004), and several health conditions has been associated with steady or regular intake of coca cola (Amato *et al.*, 1997). There is some evidence that consumption of two Coca-colas per day can cause kidney disease (Saldana *et al.,*2007) The consumption of sugary sweetened beverages has been found to increase the rate of insulin resistance in adolescent (Kondaki *et al.*, 2013). This

insulin resistance is known to increase oxidative stress which can exert a negative influence on sperm motility (Park *et al.*, 2009; Chen et al., 2013). Caramel which is also used as a coloring in soft drinks, is composed of carefully controlled heat treatment of carbohydrate, generally in the presence of acids and alkalis in a process called Caramelitization it has been linked to also cause increase in insulin resistance and inflammation (Gaby, 2005; Vlassara *et al.,*2002). Coca-cola drink is widely consumed regularly, because of their sweet taste without knowing the toxic effects which it might cause to our health or body. According to epidemiological study regular intake of coke is associated with liver diseases, tooth decay and type 2 diabetes (Adjene *et al*., 2010; Amato *et al.,* 1997) and Type 2 diabetes in adult also has been associated with lower sperm motility (Echavarria *et al.*, 2007; Ramaraju *et al.*, 2012). Increase intake of sugary drinks over the years have brought about increase in chronic kidney disease (cardiovascular risk factors such as Hypertension, diabetes, obesity and dyshpidemia are connected with the development of chronic kidney disease), especially drinks with high fructose corn syrup It was estimated that the consumption of sugar was around 68 kg (150 lb) per person per year in the US in 2003 (Fox *et al.,*2004; Johnson *et al.,*2007). This increased consumption of sugar- sweetened soft drinks has also been hypothesized to be associated with a modest but significant increase in risk among women who have an underlying degree of insulin resistance (Schernhammer *et al,* 2005), and also affect hepatic steatosis, lipid metabolism (Gaby, 2005). Recent studies have also shown that the consumption of soft drinks, and sweetened fruit soups leads to a greater risk of pancreatic cancer (Larsson *et al.,* 2006). Low sperm count can also result due to regular intake of soft drink and beverages that are high in sugar because soft drink and beverages that contains high sugar have been linked by many authors to weight gain and obesity and can lead to serious chronic diseases in adult, further research have revealed up to 25 separate harmful effects excess consumption of soft drinks can cause to our body system, A recent study in rodents also found that sugary drinks can have negative impact in male fertility (Amato *et al.*,1997; Wright *et al.,* 2001; Malik *et al.*, 2010, Mozaffarian *et al.,* 2011, and Pan *et al.,* 2013). In addition to the high sugar content, Cola beverages also contain phosphoric acid which is colorless, odorless crystalline liquid. It gives coca cola a sharp flavor and prevents the growth of mold and bacteria, which can multiply easily in sugary solution (Saldana *et al.,*2007), phosphorous may have an effect in the kidney causing kidney dysfunction , laboratory studies have shown that high phosphorous diets can cause nephrocalciosis in rats (Matsuzaki *et al.*, 1997). It has also been associated with urinary changes that promote kidney stones (Shuster *et al.,*1992). Increase in phosphate level may increase plasma phosphorous levels, with phosphate in colas perhaps

being more bioavailable. (Calvo *et al.,* 2003; Uribarri*et al.,*2003). The aim of this study is to assess the relationship between the drinking of coke and the reduction in sperm count, determine the effect of coca cola on renal functions and evaluate the effects of a daily dose on the liver and kidney.

MATERIALS AND METHODS

Experimental Design:

Twenty four(24) male Albino wistar Rats weighing between 175-250 gram were used for the study, the rats were divided into five (5).Group 1(0ml) comprises the control group, they were fed with food and water, no treatment on them, Group 2 were treated with 2ml of coca-cola using a 2ml syringe. Group 3 were treated with 1.8ml of coca-cola using a 2ml syringe. Group 4 were treated with 1.9ml of coca-cola using a 2ml syringe, Group 5 were treated with 1.8ml of coca-cola using a 2ml syringe and the albino rats were acclimatized for seven days before treatment and the administration of coca-cola was done orally.

Biochemical analysis:

Standard procedures were ensured during the collection of the blood, sperm and liver samples prior to biochemical analysis. The epididymal sperm count was determined with the Neubauer haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) and light microscope at 40× magnifications. The plasma activity of Alkaline Phosphatase (ALP) was determined using Radox kit (colorimetric method) of Rec (1972). Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen (Flack and Woollen, 1984). The plasma activity of aspartate transaminase AST and alanine transaminase ALT was determined using Reitman and Frankel method (Reitman and Frankel, 1957). The serum electrolytes were determined using ISO 4000 Automated electrolyte analyser. SFRI, France.

Method of Data Analysis

Data were analyzed using Tukey test at a level of 5% probability, using Assitat Software Version 7.7 en (2017).

RESULTS

Effects of Coca-cola on Haematology of Albino rat.

The result in Table 1 showed that on the first week, PCV had a mean value of 33.67, 37.16, 35.6 and 32.7 for the treated group for Week 1, week 2, week 3 and week 4 respectively while the control had a mean of 26.67, 32.57, 32.85 and 39.06 for week 1, week 2, week 3 and week 4 respectively with an average control of 30.69. Only week 4 had a significant difference ($P<0.05$) but there was no significant difference ($P>0.05$) across the week. Hb for control group had a mean value of 9.0, 9.9, 10.03 and 13.86 for week 1, 2, 3 and 4 respectively, and the treated group had a mean value of 11.2, 11.26, 11.25 and 13.4for week 1, 2, 3 and 4 respectively. There was a significant difference across the week ($P<0.05$) and no significant difference within the week ($P>0.05$). RBC and WBC had a mean value of 4.23 and 5.26 in week 1, 5.56 and 12.56 in week 2, 6.04 and 14.56 in week 3 and finally 6.9 and 11.9 in week 4 in the treated group while the control group had a mean value of 4.76 and 9.0 in week 1, 7.31 and 9.86 in week 2, 6.35 and 7.46 in week 3, and week 4 had 6.3 and 6.26 with an average control of 5.27 and 8.15. There was also a significant difference ($P<0.05$) across the week for both RBC and WBC while only WBC had a significant difference ($P<0.05$) within week 4. Platelet and lymphocyte had a mean value of 315 and 70 in week 1, 733 and 83.67 in week 2, 383 and 83.76 in week 3 and finally 419 and 82.26 in week 4 in the treated group while the control group had a mean value of 270 and 70 in week 1, 335.67 and 84.4 in week 2, 423 and 78.2 in week 3, and week 4 had 416.67 and 84 with an average control of 343 and 77.53. There was also a significant difference ($P<0.05$) across the week for both Platelet and lymphocyte while platelet was significantly different ($P<0.05$) in week 2 and Lymphocyte.

Effect of Coca-cola on liver, kidney and Semen of Albino rat.

The results in Tables 2 and 3 for the kidney analysis, Na had a mean value of 142, 140, 133.6 and 141.66 for the treated group in week 1, 2, 3 and 4 respectively. While the control group had a mean value of 133.67, 157.67, 136.67 and 149.67 with an average control of 147.3. K and Cl in the treated group had a mean value of 5.2 and 93.0 in week 1, 4.8 and 94.67 in week 2, 5.6 and 108.66 in week 3 with 5.2 and 107.67 in week 4. The control group had a mean value of 4.06 and 100.67 for week 1, 7.26 and 109.67 in week2, 5.0 and 120.0 in week

3 and week 4 had 5.1 and 107.67 with an average control of 5.4 and 99.33 respectively. Bicarbonate had a mean value for week 1, 2, 3 and 4 as 22.0, 24.0, 28.0 and 18.67 respectively for the treated group, and 23.67, 23.6, 24.67 and 23.0 for week 1, 2, 3, and 4 in the control group with no significant difference ($P>0.05$) across the week. The AST and ALT had a mean value of 20.67 and 10.0 in week 1, 23.0 and 9.0 in week 2, 31.67 and 13.67 in week 3, 31.67 and 13.0 in week 4 with the control having a mean value of 17.67 and 10.67 in week 1, 34.67 and 10.0 in week 2, 24.0 and 11.0 in week 3, and 23.0 and 13.0 in week 4 with an average control of 25.67 and 10.67. There was no significant difference ($P>0.05$) across the week for both AST and ALT, while only week 4 in AST had a significant difference ($P<0.05$) within the week. Protein had a mean value of 81.83, 65.8, 54.35 and 73.24 in week 1, 2, 3 and 4 respectively in the treated group. While the control had a mean value of 65.7, 72.31, 69.26, and 73.27 in week 1, 2, 3 and 4 respectively. The average control was 69.11. The sperm count analysis carried out showed that week one, two, three and four, had significant difference in the control when compared with the test at ($P<0.05$) with a mean value of 425, 140, 325 and 400 from week 1,2, 3 and 4 respectively with a control of mean value 650, 465, 575 and 575 and an average control of 566.67.

Table 1: Effects on Hematological Parameters in rats treated orally with coca cola (coke) for7 days, 14 days, 21 days and 21 days + 7 days withdrawal.

	Treatment	Treatment	PCV (%)	Hb (g/dl)	RBC($X10^{12}$)	WBC($X10^{9}$)	PLATELET	LYMPH. ($X10^{9}$)
Week 1	7 Days	Control	26.67±1.52 [a]	9.0±0.3 [a]	4.76±0.25 [a]	9.0±2.5 [a]	270.0±0 [a]	70.0±5 [a]
		test	33.67±4.5 [a,A]	11.2±1.5 [a,AB]	4.23±0.95 [a,B]	5.26±0.75 [a,B]	315.0±35 [a,B]	70.0±0 [a,B]
Week 2	14 days	Control	32.57±2.95 [a]	9.9±0.9 [a]	7.31±0.7 [a]	9.86±5.65 [a]	335.67±105.5 [b]	84.4±1.4 [a]
		Test	37.16±3.75 [a,A]	11.26±1.15 [a,AB]	5.56±0.29 [b,A]	12.56±5.05 [a,AB]	733.0±96 [a,A]	83.67±7.5 [a,AB]
Week 3	21 days	control	32.85±3.95 [a]	10.03±1.15 [a]	6.35±0.64 [a]	7.46±2.85 [a]	423.0±108 [a]	78.2±1.4 [b]
		Test	35.6±0.9 [a,A]	11.25±0.35 [a,AB]	6.04±0.43 [a,AB]	14.56±3.75 [a,A]	383.67±53 [a,B]	83.76±1.35 [a,A]
Week 4	21 days+ 7 days withdrawal	Control	39.06±2.35 [a]	13.86±0.45 [a]	6.30±1.67 [a]	6.26±0.05 [b]	416.67±3.5 [a]	84.0±0.7 [a]
	Weekly average	Test	32.7±1.22 [b,A]	13.4±0.73 [a,A]	6.90±0.1 [a,AB]	11.90±1.3 [a,AB]	419.33±7.7 [a,B]	82.26±1.95 [a,AB]
	control	control	30.69±2.81[A]	9.75±O.78[B]	5.27±0.53[B]	8.15±3.6[B]	343.0±71.17[B]	77.53±2.6[AB]

[a-b] Different letters in the same column indicate significance difference ($p<0.05$) within the week

[A-B]Diffferent letters in the same column indicate significance difference ($p<0.05$) across the week

Table 2: Effects on Liver and Renal function in rats treated orally with coca-cola (coke) for 7 days, 14 days, 21 days and 21 days + 7 days withdrawal.

	Treatment	Treatment	**Na** (mmol/l)	**K** (mmol/l)	**Cl**(mmol/l)	**Bicarbonate**(mmol/l)	**AST** (U/L)	**ALT** (U/L)	**PROTEIN**
Week1	7 days	Control	133.67±2.51^{b}	4.06±0.25^{a}	100.67±4.5^{a}	23.67±0.57^{a}	17.67±3.51^{a}	10.67±1.52^{a}	65.7±12.1^{a}
		Test	142±3^{a,A}	5.2±0.7^{a,A}	93.0±7^{a,A}	22.0±2.00^{a,AB}	20.67±6.51^{a,A}	10.0±2^{a,BC}	81.83±11.8^{a,A}
Week 2	14 days	Control	157.67±22.5^{a}	7.26±2.55^{a}	109.67±18.5^{a}	23.6±1.52^{a}	34.67±3.51^{a}	10.0±2^{a}	72.31±3.36^{a}
		Test	140.67±1.52^{a,A}	4.80±0^{a,A}	94.67±2.52^{a,A}	24.0±3^{a,AB}	23.0±1.00^{b,A}	9.0±1^{a,C}	65.8±0.61^{b,AB}
Week 3	21 days	Control	136.67±10.5^{a}	5.0±0.6^{a}	120±4.5^{a}	24.67±3.51^{a}	24.0±5.50^{b}	11.0±4^{a}	69.26±2.15^{a}
		Test	133.6±0.5^{a,A}	5.6±0.1^{a,A}	108.66±0.5^{a,A}	28.0±0^{a,A}	31.67±2^{a,A}	13.67±0.5^{a,A}	54.35±1.15^{b,B}
Week 4	21 days+ 7days withdrawal	Control	149.67±0.5^{a}	5.1±0.1^{a}	106.0±1^{a}	23.0±1^{a}	23.0±1^{b}	13.0±1^{a}	73.27±2.15^{a}
		Test	141.66±0.47^{b,A}	5.2±0.08^{a,A}	107.67±1.25^{a,A}	18.67±2.86^{a,B}	31.67±0.47^{a,A}	13.0±0.82^{a,AB}	73.24±0.82^{a,A}
	Weekly average control	Control	147.3±11.8^{A}	5.4±I.12^{A}	99.33±9.17^{A}	24.3±1.8^{AB}	25.67±4.17^{A}	10.67±1.3^{ABC}	69.11±5.9^{A}

$^{a-b}$ Different letters in the same column indicate significance difference ($p<0.05$) within the week

$^{A-B}$Diffferent letters in the same column indicate significance difference ($p<0.05$) across the week

Table 3: Effect on Sperm Count in rats treated orally with coca-cola (coke) for 7 days, 14 days, 21 days and 21 days + 7 days withdrawal.

	Treatment	Treatment	Sperm Count($x10^6$)
Week 1	7 days treatment	Control	650±50 [a]
		Test	425±108.3 [a,AB]
Week 2	14 days treatment	Control	465±175 [a]
		Test	140±225 [b,B]
Week 3	21 days treatment	Control	575.0±25 [a]
		Test	325.0±81.8 [b,AB]
Week 4	21 days treatment+ 7 days withdrawal	Control	575.0±125 [a]
		Test	400.0±0 [b,AB]
	Weekly average control	Control	566.67±83.3[A]

[a-b] Different letters in the same column indicate significance difference ($p<0.05$) within the week

[A-B]Different letters in the same column indicate significance difference ($p<0.05$) across the week

DISCUSSION

The RBC was generally lower than the Control for week 1, 2, and 3 while the week 4 which is the 7 days after withdrawal was higher than the control although not significantly. This result for RBC shows that Coca-cola exerted a negative effect on the RBC and when it was withdrawn, the body system recovered. The level of PCV was generally higher in the treated group when compared to the control group. This implies that Coca-cola causes an increase in the blood PCV although not significantly. The Hb level was observed to be significantly high

in the treated group. According to a study, abnormal high level of HB could be as a result of dehydration and kidney tumor among other effect (Fox, 2002). This can be due to the excessive consumption of Colas because reports have linked chronic kidney diseases to the consumption of two or more Colas daily (Bonnie, 2017; Axe, 2018). The WBC also had an abrupt increase in the second week up to the fourth week, with a significant difference ($p<0.05$). The result of this work is in line with the, findings in other studies of increases in WBC corresponding with increased dosage of Cola acuminate methanoic extract, (Adam *et al*.,2011; Drugnon *et al*.,2010; Bassini *et al*.,2007) and contradicts the report of (Obidike *et al*.,2011) that the extract of kola nut did not have a significant effect on rats administered 200 mg/kg of Cola acuminate methanoic extract, from WBCs count of rats. The platelet level was high in the first two weeks while the last week was low in the treated group showing that Coca-cola has negative effect on blood platelet. The abnormal and irregular rise and fall in serum electrolytes are indicators of kidney diseases which affect the ionic balance (Dhondup and Qian, 2017) and Cola beverages contains phosphoric acid and have been linked to promote kidney stones (Shuster *et al.,*1992) and also kidney dysfunction, laboratory studies have also shown that high phosphorous diets can cause nephrocalciosis in rats (Matsuzaki *et al.*, 1997). The AST level was observed to be high in the treated group compared to the average control, while ALT was high in the last two weeks when also compared to the average control and this indicates possible liver damage (Green and Flamm, 2002), and many studies have revealed that soft drinks such as Coca-cola cause Fatty Liver disease (Byrne, 2017). The sperm count was significantly low in the treated group when compared to the control group, this low sperm count decreases fertility which is in agreement with (Ruff *et al.*, 2013).

CONCLUSION

Excessive consumption of Coca-cola should be avoided due to its negative impact on the kidney, sperm and liver of rats as observed in this study.

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