Original Research Article

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

Abstract

The effect of a daily consumption of soft drink was evaluated using 24 Albino rats divided into two groups viz:control and treatment. The experiment was carried out for four (4) weeks. The treatment (a brand of soft drink) was administered to the test group for three weeks while on the fourth week no treatment was given to the test group. 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 (mmol/l) *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* (mmol/l) *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* (mmol/l) *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* (mmol/l) *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* (U/L) *mean value was 20.67 in week 1 which increased to 31.67 in week 4 while ALT* (U/L) *mean value was 10 in week 1 which also increased to 13 in week 4. The mean serum protein* (g/dL) *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* (g/dL) *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* ($X10^9$) *increased from a mean 5.26 in week 1 to 11.9 in week 4 with a significant difference (P<0.05), Platelet* ($X10^9$) *mean value was 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* ($X10^{12}$) *increased from a mean of 4.23 in week 1 to 6.90 in week 4 with significant difference (P<0.05). Lymphocyte* ($X10^9$) *mean value for week 1 was 70 and 82.26 in week 4 with significant difference (P<0.05) across the week. While the mean sperm count* ($X10^6$) *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 soft drink had a detrimental effect on the sperm count, liver, kidney and on the haematological parameters.*

INTRODUCTION

Coca-cola and Pepsi are some of the world's favorite soft drinks, they comprise of kola known to be a key source of caffeine, other components include: phosphoric acid, sugar in the form of glucose and other forms of chemicals that are used for preservation, flavor and colorings [1]. The intake of soft drinks has increased in the past two decades [2], and several health conditions has been associated with steady or regular intake of soft drinks [3]. There is some evidence that consumption of two bottles of soft drinks per day can cause kidney

disease [4]. The consumption of sugary sweetened beverages has been found to increase the rate of insulin resistance in adolescent [5]. This insulin resistance is known to increase oxidative stress which can exert a negative influence on sperm motility [6; 7]. 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 also been linked to increased insulin resistance and inflammation [8; 9]. Soft drinks are widely consumed regularly, because of their sweet taste, in many cases the consumption is without knowledge of the possible detrimental effects to our health or body if consumed daily. According to epidemiological study regular intake of soft drinks is associated with liver diseases, tooth decay and type 2 diabetes [1,3] and Type 2 diabetes in adult also has been associated with lower sperm motility [10, 11]. It was estimated that the consumption of sugar was around 68 kg (150 lb) per person per year in the US in 2003 [12, 13]. 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 [14], and also enhance hepatic steatosis [8]. Recent studies have also shown that the consumption of soft drinks, and sweetened fruit soups leads to a greater risk of pancreatic cancer [15]. A recent study in rodents also found that sugary drinks can have negative impact in male fertility [3, 16, 17, 18 and 19]. In addition to the high sugar content, Cola beverages also contain phosphoric acid which is a colorless, odorless crystalline liquid. It gives the soft drink a sharp flavor and prevents the growth of mold and bacteria, which can multiply easily in sugary solution [4], phosphorous may have an effect on the kidney causing kidney dysfunction, laboratory studies have shown that high phosphorous diets can cause nephrocalciosis in rats [20]. It has also been associated with urinary changes that promote kidney stones [21]. Increase in phosphate level may increase plasma phosphorous levels, with phosphate in colas perhaps being more bioavailable. [22, 23]. This study therefore aims at assessing the effect of daily consumption of cola soft drinks on sperm count, determine their effect 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 grams were used for the study, they were acclimatized for seven days before any treatment. An average weight adult human of 65kg drinks about 350ml of soft drink, this body weight was used to estimate

the concentration in millilitres administered to the rats based on their body weight. The daily dose administered was based on the weekly body weights of the rats. The rats were divided into two (2) groups. Group 1comprised the control group, they were fed with regular feed and water, no treatment was administered to them. Group 2 the treatment group had daily access to drinking water and feed and were treated with 1ml to 1.3ml of soft drink depending on their weekly body weight using a 2ml syringe through the oral route. The experiment was carried out for four (4) weeks. The treatment (a brand of soft drink) was administered to the test group daily for three weeks while on the fourth week no treatment was given to the test group. This was done to observe their possible recovery from any effects of the treatment. Three (3) rats of uniform weight from the test group were sacrificed weekly and three (3) rats from the control group were sacrificed weekly. This was done to enable us to collect blood and sperm samples for analysis. The animals were sacrificed by jugular puncture while under anaesthesia. Blood samples collected were taken with both EDTA and Heparin bottles for laboratory analysis while the testes were collected for sperm analysis which was done using an electron microscope.

Biochemical analysis:

Standard procedures were ensured during the collection of the blood and sperm 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. Haemoglobin, Packed Cell Volume, White Blood Cells, Red blood cells, Platelets and lymphocyte counts were determined according to the methods of [24]. Electrolytes were determined according to the methods of [25]. The plasma activity of Alkaline Phosphatase (ALP) was determined using Radox kit (colorimetric method) of [26]. Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen [27]. The plasma activity of aspartate transaminase AST and alanine transaminase ALT was determined using Reitman and Frankel method [28]. 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 Soft drink on Haematology of an Albino rat

The result in Table 1 shows the summary of effect of Soft drink on some blood parameters; it shows the mean value and Standard Deviation (STDEV) for each of the parameters. The result for Red Blood Cell (RBC), Packed Cell Volume (PCV), and Hemoglobin (Hb), in rats treated with Soft drink for 7 days (week 1) showed that there was no significant difference ($p>0.05$) compared to the control, while for White Blood Cell (WBC), Platelet, and Lymphocytes, there was also no significance difference($p>0.05$). PCV, Hb, WBC, and Lymphocytes showed no significant difference ($p>0.05$) in rats treated with Soft drink orally for 14 days (2^{nd} week) while RBC and Platelet had a significant difference ($P<0.05$) when compared to the control. When the treated group after 21 days (3^{rd} week) were compared to the control, PCV, Hb, RBC, WBC and Platelet had no significant difference ($P>0.05$) while only Lymphocytes had a significant difference ($P<0.05$). PCV and WBC showed significant difference ($p<0.05$) in rats treated with Soft drink for 21 days + 7 days withdrawal (4^{th} week) with Hb, RBC, Platelet and Lymphocytes having no significant difference ($P>0.05$) compared to the control. The result also showed non-significant differences ($p>0.05$) in PCV, Platelet and Hb in rats treated with soft drink orally for 7 days, while RBC, WBC and Lymphocytes showed significant difference ($p<0.05$) in rats treated with soft drink orally for 7 days, compared to weekly average control. The treated group showed no significant difference ($p>0.05$) in Hb, RBC and WBC in rats while PCV, Platelets and Lymphocytes had a significant difference ($P<0.05$) for 14 days compared to weekly average control. After 21 days, only Platelets had no significant difference ($P>0.05$) while PCV, Hb, RBC, WBC and Lymphocyte had a significant difference ($P<0.05$) when comparing the treated group with the average control. The treatment effect on Lymphocyte showed non-significant difference ($p>0.05$) in rats treated with Soft drinkorally for 21 days+ 7 days withdrawal while there were significant differences ($P<0.05$) in PCV, Hb, RBC, WBC and Platelet of treated rats compared to the control.

Effect of Soft drink on liver, and kidney of Albino rat

The result in Table 2 shows the summary of effect of soft drink on kidney and liver parameters evaluated. Chlorine (Cl), Alanine Aminotransferase (ALT), Bicarbonate, Aspartate Aminotransferase (AST) and potassium (K) were non-significantly different ($p>0.05$) while Sodium (Na^+) recorded a significant difference ($P<0.05$) in rats treated with soft drink orally for 7 days compared to their control. Only AST and Protein showed significance difference ($p<0.05$), in rats treated with soft drink orally for 14 days and 21 days, compared to the control. The rats after 21 days+ 7days withdrawal recorded a significant difference ($P<0.05$) in Sodium and AST only when comparing the treated group with the control. Na^+, ALT, AST, CL, Protein, Bicarbonate and K^+ showed non-significance difference ($p>0.05$) in rats treated with soft drink orally for 7days, compared to average weekly control. In week 2 (14 days), all the parameters had no significant difference ($P>0.05$) when compared to the control, week 3 (21 days) had a significant difference ($P<0.05$) only in Protein. Week 4 (21 days+ 7 days withdrawal) had a significant difference ($P<0.05$) only in ALT when compared to the weekly average control.

Effects of Soft drink on Sperm Count

The result in Table 3 shows the summary of effect of soft drink on sperm count. There were no significant difference ($p>0.05$) in sperm count of rats treated with soft drink orally for 7days and the control. Significant differences ($P<0.05$) in sperm count were observed when comparing the treated group with the control after 14 days, and 21 days treatments. Treatment also showed significant difference ($P<0.05$) in rats treated with soft drink orally for 21 days + 7 days withdrawal, compared to the control. Generally there were non-significance differences in sperm counts of rats treated with soft drink orally for 7days while a significant difference ($P<0.05$) was recorded 14 days, 21 days and 21 days + 7 days withdrawal, when compared to the average weekly control.

Table 1: Effects on Hematological Parameters in rats treated orally with soft drink 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}
		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}
	Weekly average 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

176 Table 2: Effects on Liver and Renal function in rats treated orally with soft drink 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}

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178 $^{a-b}$ Different letters in the same column indicate significance difference ($p<0.05$) within the week

179 $^{A-B}$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 soft drink 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 count 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 soft drink 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. 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 [29]. 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 [30, 31]. 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, [32, 33, 34] and contradicts the

report of [35] that the extract of kola nut did not have a significant effect on WBCs count of rats. The platelet level was high in the first two weeks while the last week was low in the treated group indicating that soft drink had a 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 [36] and Cola beverages contains phosphoric acid which is known to promote kidney stones [21] and also kidney dysfunction. Laboratory studies have also shown that high phosphorous diets can cause nephrocalciosis in rats [20]. 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 [37]. A study by [38] revealed that soft drinks may cause fatty liver disease. The sperm count was significantly low in the soft drink treated group when compared to the control group, this low sperm will affect fertility and may be due to hormonal changes associated with sugary drinks consumption and oxidative stress induced by insulin resistance [6, 7, 39 40].

CONCLUSION

This study clearly indicates that a daily dose of soft drinks had negative effects on parameters studied in rats which are mammals. Since the primary consumption of soft drinks is by man belonging to the class mammalia having similar though higher and more advanced anatomical and physiological responses with rats, Excessive consumption of soft drinks should be avoided due to its negative impact on the kidney, sperm and liver as observed in this study.

ETHICAL APPROVAL

As per international standard or University standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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