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Risks of a fructose rich soft drink consumption on some biochemical parameters in Balb/c mice

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ABSTRACT

Consumption of soft drinks, in particular carbonated beverages, has markedly increased in the last two to three decades. In fact, the carbonated beverages are the most popular refreshments among most of the world's population. The aim of this study was to assess the body weight gains and analyze some biochemical and hematological parameters in male and female Balb/c mice. Animals were provided with diluted coca cola (fructose –rich soft drink) for 6 weeks and were matched against corresponding control animals. The control animals were maintained on tap water *ad libitum* and coca cola was diluted v/v by tap water. The results revealed that the percentages of body weight gains of male and female control animals were significantly higher than the corresponding mice provided with coca cola. Hemoglobin content (Hb, g/100ml blood), erythrocytes (RBCs), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), leucocytes (WBCs), granulocytes and blood platelet counts were significantly decreased in the groups of mice treated with the soft drink, compared to the control groups. However, significant increases were observed in lymphocytes in the treated groups. Consumption of the soft drink (coca cola) in both male and female animal groups exhibited significant increases of total cholesterol, triglycerides, low density lipoprotein-c and atherogenic index. Levels of serum calcium significantly decreased in both male and female treated mice than the corresponding control groups. Consumption of coca cola did not affect the levels of T_3. This study suggests that consumption of high fructose soft drink may lead to many health problems.

Key words: *coca-cola beverage- lipid profile- blood picture – triiodothyronine-calcium*.

INTRODUCTION

A global change in dietary habits has occurred over the last few decades resulting from the introduction of sweeteners such as fructose and sucrose by the food industries. Consumption of soft drinks and fruit drinks, the major sources of high fructose corn syrup (HFCS) or sugar, have increased from 3.9% of the total energy intake in 1977 to 9.2% of the total energy intake in 2001[(1)].

In Egypt, sales of carbonated soft drinks reached 1,650 million liters after average growth of 13% per annual over the past 5 years. Carbonated soft drinks are consumed by all sections of society, especially children. Although consumer researches apparently suggest that consumers are aware of "health" issues surrounding carbonated soft drinks, it does not seem to be impacting consumption levels (www.drink expo.ae/marketing.php.). Several factors may be associated with the high consumption of soft drinks such as taste preference, habits of parents and friends, soft drink availability in home and school and television's advertisements.

The soft drink usually contains the following components: phosphoric acid, caffeine, sugar or aspartame or saccharin, caramel colour, carbon dioxide, and natural flavoring [(2)]. Intake soft drinks do not cause any immediate warning such as stomach cramps, vomiting, or diarrhea instead, energizing feeling of caffeine, sweet taste of sugar combined with sour taste of phosphoric acid, and the playful feeling of the carbon dioxide bubbles. However, those ingredients cause imbalances in the body systems that result in debilitating diseases that appear after several years of abuse [(3)].

Most of the soft drinks are sweetened with sugars containing a high proportion of fructose [(4)]. Whether sucrose (50% fructose) or high fructose corn syrup (usually 55% fructose) is used as the sweetening agent, the fructose content of beverages sweetened with sugars ranges from 7% to 15% by weight [(5)]. Increase of fructose consumption has simultaneously associated with the dramatic raise in prevalence of obesity [(6&7)].

Unlike glucose, which is metabolized in every body tissue, fructose is primarily metabolized in the liver and poorly stimulates insulin and leptin secretion [(8)]. While the metabolism of glucose is negatively regulated by phosphofructokinase, fructose can continually enter the glycolytic pathway, leading to a high production of pyruvate and providing hydrocarbons for glycerol and triglyceride synthesis. The resulting excess in intracellular energy

flux is associated with insulin resistance, stimulation of inflammatory pathways, ApoB production, and uncontrolled triglyceride synthesis that together lead to hepatic stress [9].

Carbonated drinks with high sugar content have been reported to have no nutritional value [10]. Consumption of carbonated beverages negatively correlated with nutrients of calcium, magnesium and vitamins B1, B2, B3, B6, B12 in addition to vitamin C [11]. In fact, the adolescent growth period is a critical time for bone mineral accretion but, the intake of carbonated beverages increased bone fractures during the adolescence [12]. Moreover, a significant relationship between consumption of carbonated beverages containing phosphoric acid (Coca-Cola and Pepsi-Cola) and hypocalcemia was found among children of 2.4-5.6 years old [13]. Furthermore, teeth decay is a consequent of high intake of sugar and soda [14].

It is worth mentioning that, to the best of our knowledge, there are scanty literatures concerning risks of high consumption of the national manufactured soft drinks (the Egyptian product). Therefore, the aim of this work was to study the effect of high consumption of the Egyptian product of coca cola for 6 weeks on hematological and some biochemical parameters of male and female Balb/c mice.

MATERIAL AND METHODS

Animals

Forty male and female Balb/c mice weighing 15-18g, 21 days old, were housed in plastic cages located in the animal house of Nuclear Research Center, Inshas, under the same environmental conditions.

Experimental groups

The animals were divided into two main groups each was subdivided into male and female subgroups (each of 10 mouse): Group (1), served as control groups, animals were maintained on tap water, ad libitum. Group (2), treated groups, animals were provided with the soft drink coca cola, purchased from the local markets in Egypt, diluted v/v with tap water. Constant volumes of diluted cola beverage were offered daily and the consumptions/day/mouse was estimated (3 ± 1.5ml/day/mouse). The mean body weights/cage/week was also calculated. All animals were sacrificed after six weeks by decapitation. Blood samples were collected into plain tubes and tubes with EDTA. Blood with EDTA were used for investigating blood picture and the plain blood was

centrifuged at 3000rpm for 15 minutes to obtain serum. Due to the limited blood volume of each mouse, serum samples of each two mice were pooled for the following biochemical analysis: lipid profile, calcium and triiodothyronine.

Hematological investigation

Total counts of erythrocytes (RBCs) and leucocytes (WBCs) were performed using an improved Neubauor chamber. Differentiations of white blood cells were carried out according to Carleton [15].

Hemoglobin content (Hb by g/100ml blood) was determined using Hemoglowiner Laboratory Kit and the resulting cyanmethemoglobin was measured spectrophotometrically at 546nm. The hematocrit value (HCT) was determined in duplicate samples by microhaematocrit centrifuge at 3000 rpm for 15 minutes. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and blood platelet counts were estimated according to Dacie and Lewis, [16].

Biochemical analyses

Serum triglycerides (TG), total cholesterol (TC), High Density Lipoprotein-Cholesterol (HDL) and Low Density Lipoprotein-Cholesterol(LDL) were analyzed by means of colorimetric enzymatic methods (Laborlab kits), using PRIME-E automatic digital photometer at wave length 505nm. Very low density lipoprotein cholesterol (VLDL-c) was calculated according to Lee and Nieman [17] as follows: VLDL-c = TG / 5

Atherogenic index was calculated by dividing LDL-c by HDL- according to Castelli and Levitar [18].

Calcium was performed using SPIN React kit and measured spectrophotometrically at 570nm.

Serum levels of (3,3,5 triiodothyroxin T_3) were carried out using radioimmunoassay commercial kits (Institute of Isotopes Co., LTD Budapest).

Statistical analyses

The data were expressed as means ± standard deviations. Two-way analysis of variance was performed among the mean values of the groups followed by Duncan's multiple range test, whenever necessary . The statistical difference considered significant at $P<0.05$. All statistical analyses were performed using COSTAT.

RESULTS

Body weight

The body weights, in grams, are represented as means ± standard deviations for the various tested groups in Table (1).

The increases in the body weights of the control male and female animals were significantly higher in the control animals than the increase in the body weights of the corresponding treated animals. The percentages of the body gains reached 58.9 and 60.7 in both male and female animals maintained on tap water, compared to 22.7 and 35.8 of male and female mice, respectively, treated with coca cola (Fig. 1& Table 1). Significant differences between males and females were also shown.

Hematological parameters

Table (2) shows the effect of coca cola consumption on the hematological parameters of male and female Balb/c mice. Hematological investigation showed that Hb content, RBCs count, HCT, MCV and MCH were significantly decreased in the groups of male and female mice treated with the soft drink, compared to the corresponding control groups. Significant decreases were also observed in blood platelets, WBCs count and granulocytes in both sexes of the treated groups, compared to the corresponding control groups. However, significant increases were observed in lymphocytes in the treated groups above the corresponding control groups. No changes of MCHC were observed.

Lipid profile

Table (3) shows the effect of coca cola intake on serum TC, TGs, HDLc and LDLc levels. Consumption of the soft drink coca cola exhibited significant increases of TC, TGs, LDLc and VLDLc concentrations in both male and female animals, compared to the corresponding control groups.

Serum calcium

Table (4) shows mean values of serum calcium levels among the different groups. The mean values of serum calcium in both male and female mice maintained on coca cola decreased significantly, compared to the control groups.

Serum triiodothyronine (T_3)

Table (4) also shows the mean values of serum T_3 levels of the tested groups. Non-significant increases were observed after coca cola consumption in both male and female mice.

Table (1): Effect of coca cola on the development of body weights of male and female Balb/c mice.

Groups / Body weights	Control		Coca cola	
	Male N=10	Female N= 10	Male N=10	Female N=10
Initial body weights (g)	13.7 ± 1.1 [a]	14.4 ± 0.9 [a]	13.4 ± 1.9 [a]	14.8 ± 2.6 [a]
Final body weights (g)	21.7 ± 2 [ab]	23.1 ± 1.5 [a]	16.54 ± 3.2 [c]	20.3 ± 3.5 [b]
Body weight gains % after6 weeks	58.9 ± 3.8 [a]	60.7 ± 4.6 [a]	22.7 ± 10.1 [c]	35.8 ± 13.3 [b]

Values are shown as means ± SD of N=10.

Same letter in the same row indicate non significant difference between groups

Different small letters in the same row indicate significant difference between groups at P< 0.05.

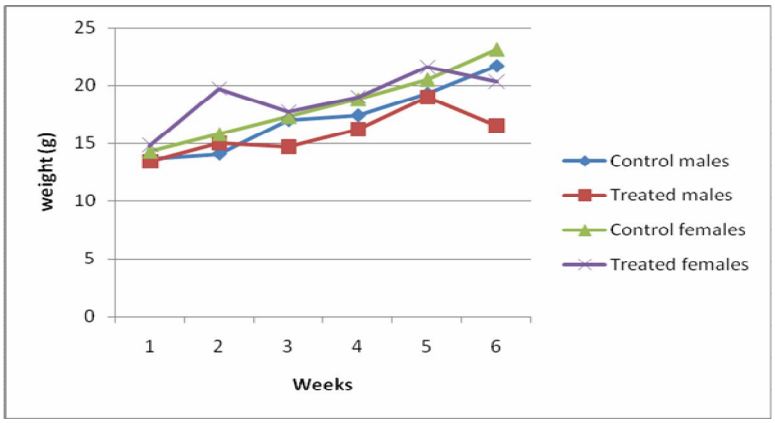

Fig. (1): Effect of coca cola on changes in the body weights of male and female Balb/c mice throughout 6 weeks.

Table (2): Effect of coca cola on hematological parameters in male and female Balb/c mice.

Groups / Parameters	Control		Coca cola	
	Male	Female	Male	Female
Hb (g/dl)	16.2 ± 0.4 [a]	15.1 ± 0.9 [b]	13.9 ± 0.7 [c]	12.3 ± 0.9 [d]
RBCs $\times 10^6/mm^3$	10.04 ± 0.5 [a]	9 ± 0.4 [b c]	9.4 ± 0.7 [b]	8.4 ± 0.4 [c]
HCT %	48.4 ± 2.6 [a]	45.2 ± 2.6 [b]	42.6 ± 2.2 [c]	36.9 ± 2.8 [d]
MCV μm^3	48.3 ± 2.6 [a]	50.3 ± 3.5 [a]	45.6 ± 1.7 [b]	43.8 ± 1.3 [b]
MCH (Pg)	16.1 ± 0.9 [a]	16.8 ± 1.2 [a]	14.9 ± 0.3 [b]	14.6 ± 0.4 [b]
MCHC (g/dl)	33.3 ± 0.2 [a]	33.3 ± 0.1 [a]	33.3 ± 0.3 [a]	33.3 ± 0.2 [a]
WBCs $\times 10^3/mm^3$	5.8 ± 0.6 [a]	5.7 ± 0.7 [a]	3.9 ± 0.9 [b]	3.7 ± 0.3 [b]
LYM $\times 10^3/mm^3$	1.8 ± 0.2 [c]	2.4 ± 0.3 [b]	3.2 ± 0.3 [a]	2.9 ± 0.3 [a]
MON $\times 10^3/mm^3$	0.34 ± 0.5 [a]	0.38 ± 0.1 [a]	0.28 ± 0.2 [a]	0.31 ± 0.6 [a]
GRA $\times 10^3/mm^3$	3.6 ± 0.4 [a]	2.9 ± 0.3 [b]	0.49 ± 7.6 [c]	0.47 ± 0.3 [c]
PLT $10^3/mm^3$	1099.4±115.1 [a]	993.5 ± 79.5 [b]	433.2 ± 110.2 [c]	310.4 ± 50.8 [d]

Values are shown as the means ± SD of N=10.

Same letter in the same row indicate non significant difference between groups.

Different small letters in the same row indicate significant difference between groups at P< 0.05.

Table3: Effect of coca cola on lipid profile in male and female Balb/c mice

Groups / Parameters	Control		Coca cola	
	Male	Female	Male	Female
TC (mg/dl)	119.2 ± 2.9 [b]	91.6±2.1 [c]	153±7.8 [a]	114±14.5 [b]
TG (mg/dl)	90.6 ± 3.1 [b]	82.6 ± 3.4 [b]	117 ± 10.6 [a]	120.8 ± 9.9 [a]
LDLc (mg/dl)	44.7 ± 5.7 [b]	25.5 ± 0.9 [d]	74.3 ± 4.4 [a]	35 ± 11.9 [c]
HDLc(mg/dl)	56.4 ± 4.9 [a]	49.6±1.8 [a]	55.5±3.8 [a]	52.8±6.7 [a]
VLDLc (mg/dl)	18.1 ± 0.6 [b]	16.9 ± 1.06 [b]	23.4 ± 2.1 [a]	24.2 ± 1.9 [a]
LDL/HDL ratio	0.8 ± 0.16 [b]	0.51 ± 0.02 [c]	1.3 ± 0.06 [a]	0.69 ± 0.28 [bc]

Values are shown as means ± SD of N=10

Same letter in the same row indicates non significant difference between groups.

Different small letters in the same row indicate significant difference between groups at P< 0.05.

TC, Total cholesterol; TG, Triglycerides; LDLc, Low density lipoprotein cholesterol; HDLc, High density lipoprotein cholesterol and VLDLc Very low density lipoprotein cholesterol.

Table (4): Effect of coca cola on serum calcium and T_3 hormone of male and female Balb/c mice

Groups / Parameters	Control		Coca cola	
	Male	Female	Male	Female
Serum calcium (µg/dl)	12.6 ± 1.2[a]	11.8 ± 1.8[a]	10.2 ± 1.5[b]	8.9 ± 0.5[b]
T_3 (ng/dl)	177.8 ± 3.8[a]	150.1 ± 41.8[a]	179.6 ± 4.2[a]	160.1 ± 38.8[a]

Values are shown as the mean ± SD of N=10.

Same letter in the same row indicate non significant difference between groups.

Different small letters in the same row indicate significant difference between groups at P< 0.05.

DISCUSSION

The obtained results showed that the final body weights and the percentages of body weight gains of both male and female Balb/c mice that consumed coca cola beverage were significantly lower than the corresponding control groups. This might be attributed to the short term effect of fructose (6 weeks) or to the presence of caffeine in the soft drink that might be effective in inhibiting the proliferative activity of the white adipose tissues, thereby retarding obesity [(19)]. These obtained results are in agreement with many other authors. El -Badrawy, *et al.* [(20)], showed that administered carbonated soft beverages to rats for 8 weeks showed significant decreases in final weight, weight gain and weight gain percentage between treated and control animals. Also, Botezelli, *et al*. [(21)] reported high consumption of coca cola soft drink did not affect body weight of the rats. Moreover, Bukowiecki *et al* [(22)], proved that the total energy intake of rats consuming soft drinks increased by 50% without excess weight gain. In addition, short-term effects of fructose are not correlated with body weight [(23)].

In contrary, soft drinks contain high fructose corn syrup, more than 40 % of caloric sweeteners, increased energy intake that leads to lipogenesis and hence increase body weight gain/obesity [(24&25)]. This might be due to the long term effect of fructose corn syrup (2- 7 months).

The present study showed that coca cola beverage significantly decreased Hb, RBCS, HCT, MCV, MCH, and blood platelets counts.

The significant decreases in the contents of hemoglobin in both male and female treated groups are in accordance with the significant decreases in

RBCs, HCT and MCV. The decrease in hemoglobin content might reflect the decreased rate of hemoglobin synthesis due to the presence of caffeine, as an ingredient, in coca cola. Caffeine interferes with the absorption of minerals such as calcium, magnesium, potassium, and iron. In addition, coca cola rich in fructose lead to mineral loss, especially through high fecal excretions of iron and magnesium. Iron is an essential component of red blood cells and iron deficiency contributes to develop anemia. Lewis [26] reported fall in the rate of hemoglobin synthesis during all maturation stages of erythrocytes because of inadequate iron supply. Moreover, fructose inhibits copper metabolism and a deficiency in copper leads to anemia [27].

The present study further revealed significant decreases of the RBCs count in the treated groups which are in accordance with the reduced hemoglobin content that might reflect an inhibition of erythropoiesis in bone marrow. Reduction of RBCs might be attributed to the presence of sodium benzoate as a preservative in soft drinks. Presence of sodium benzoate in soft drinks could be mixed with Vitamin C to form Benzene, a carcinogenic substance-damage bone marrow, leading to anemia and decrease in red blood cells [28]. Some authors concluded that administration of benzene results in inhibition of DNA synthesis in the bone marrow [29&30]. Recent surveys found high levels of benzene in some soft drink brands [31].

The present study also revealed a significant decrease in haematocrit percentages in the treated groups. The decrease in haematocrit could be due to the decrease in the number of RBCs or to decrease haemoglobin synthesis [32].

Reduction in MCH was also recorded in the treated groups that might reflect iron deficiency anemia [26].

The present study showed significant decreases in WBCs count in the treated groups. This might be due to the presence of benzene in soft drinks [28&29]. Benzene could damage bone marrow and inhibit the immune system [31].

Blood platelets play a major role in coronary artery disease and are found at site of early atherosclerosis. Platelets secrete potent mitogenenic factors such as platelet derived growth factor, transforming growth factor B. and epidermal growth factor which lead to smooth muscle proliferation and progression of atherosclerotic lesions. Enhanced platelet reactivity and spontaneous platelet aggregates were associated with high risk of recurrent coronary artery disease [33&34]. In the present study, consumption of coca cola

significantly decreased the blood platelet counts. Rein *et al.* [35] recommended that consumption of cola rich with polyphenols, as an additive, has a role like aspirin action in suppression of platelet aggregations and loss their functions. In addition, caffeine is weak competitive antagonists of several adenosine receptors and may contribute to the observed antithrombotic effects [36].

The obtained results revealed that consumption of the soft drink (coca cola) exhibited the higher significant increases of TC, TG, LDLc, VLDL-c concentrations and atherogenic index (LDL/HDL) in both male and female treated groups than the control groups (Table 3). This could be due to the fact that the soft drinks contain high fructose corn syrup and caffeine. These results agreed those of El-Badrawy, *et al.* [20] and Bukowiecki *et al.* [22] who reported increases in serum TC, TG, and LDL-c levels after consumption of soft beverages. Also, Bostick *et al.* [37] and Pena *et al.* [38] recorded that soft drinks, contain caffeine and refined sugar, decreased HDL-c in human being which is in accordance with the result of the males in the present work. Glass and Witztum [39] recorded increased plasma cholesterol, particularly LDL-c, is one of the most important risk factor for coronary vascular disease. LDL-c particles are taken up by macrophage cells after oxidized or modified and deposited in the arterial intimae leading to formation of atheroma.

Several authors discussed the relation between high intake of fructose and lipids. Basciano *et al.* [40] strictly investigated the metabolic pathways of animals undergoing fructose overload. They recorded both positive and negative effects of fructose consumption. Although fructose is a potent regulator of glycogen synthesis and of glucose utilization by the liver, its regulatory effects decrease with chronic intake of fructose. In addition, because of its lipogenic properties, excess fructose in the diet makes the absorption of glucose difficult and elevates circulating TG and TC. In the study of Bocarsly *et al.* [25], ingestion of fructose-based diets is characterized by metabolic dysfunctions and rapid increases in serum TG. Lim *et al.* [41] reported that fructose is absorbed in the intestine by the glucose transporter GLUT5, rapidly absorbed from the portal blood to convert into fructose-1-phosphate in liver and freely enters the glycolytic pathway. Fructose metabolism leads to an accumulation of intermediates of glycolysis that are converted to glycerol and acetyl-coenzymeA (CoA) before being synthesized into fatty acids, very-low-density lipoproteins, and triglycerides.

Data presented in Table (4) is in parallel with the results that coca cola

soft drink consumption is correlated with osteoporosis where high phosphorus content of the beverages is associated with an increase in calcium excretion. Consumption of phosphorus rich diet with low calcium content resulted in reduced serum calcium [42&43].

Phosphoric acid, a component of cola beverage, reduces 25-dihydroxyvitamin-D synthesis, interferes with intestinal absorption and reabsorption of calcium leading to hypocalcemia and secondary hyperparathyroidism [44]. In addition, low calorie soft drinks are high in sodium which increases calcium excretion in the urine and increases risk of osteoporosis [45]. Furthermore, caffeine in cola beverages has been reported to reduce bone mineral density and increase fracture risk [46].

Harris [47] and Heaney *et al.* [48] recorded that excessive use of caffeinated products increase urinary calcium excretion due to a reduction in renal tubular resorption and ultimately drain off skeletal calcium. Anonym [45] reported people who drink soft beverages instead of milk or dairy products, low calcium intake, leading to fragile and broken bones. However, and according to Kinney [49], non-cola carbonated soft drinks, do not contain phosphoric acid or caffeine has not been associated with fracture risk among children and adolescents.

The present study showed non significant increases in serum 3,3',5-triiodothyroxin (T_3) in the treated groups compared to the control groups. This might be attributed to iron deficiency where normal thyroid status is dependent on the presence of several trace elements for synthesis and metabolism of thyroid hormones [50], severe iron deficiency may inhibit thyroperoxidase activity and interfere with the synthesis of thyroid hormones [51].

CONCLUSION

The present data showed considerable negative effects of high intake of carbonated cola soft drink on body weight, hematological parameters, lipid profile and calcium levels but, the values of T_3 reflected normal thyroid function. Milk and dairy products are recommended to intake as substitutive beverages.

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مجلة البحوث الإشعاعية
والعلوم التطبيقية
مجلد ٤ عدد ٤(ب) ص ص ١٣٧٣ – ١٣٨٨ (٢٠١١)

مخاطر تناول مشروب غازي غني بالفركتوز على بعض القياسات البيوكيميائية فى الفئران

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إن استهلاك المشروبات الخفيفة خاصة المشروبات الغازية ، ازدادت بشكل ملحوظ في العقود الاثنين او الثلاثة الماضية، وتعتبر المرطبات الغازية الأكثر استهلاكا بين الكثير من شعوب العالم. الهدف من هذه الدراسة هو تحليل بعض القياسات البيوكيميائية في فئرانBalb/c تم إعطائها مشروب الكوكاكولا محلية الصنع (مشروب غنى بسكر الفواكه-الفركتوز) على مدى ٦ أسابيع. أجريت الدراسة على أربعين فأر من الذكور والإناث تم تقسيمهم إلى مجموعتين: المجموعة الضابطة (١٠ من الإناث و١٠من الذكور) أعطيت ماء فقط. والمجموعة المعاملة (١٠من الإناث و ١٠ من الذكور) أعطيت كوكاكولا مخففة بالماء بأحجام متساوية. وقد أظهرت النتائج أن الزيادة في وزن الجسم والنسبة المئوية للزيادة المكتسبة للجسم في ذكور وإناث الفئران المعاملة بالكوكاكولا اقل معنويا من الزيادة المكتسبة في المجموعة الضابطة. وأظهرت النتائج أيضا زيادة معنوية فى الكوليسترول ، الدهون الثلاثية والليبوبروتينات منخفضة الكثافة فى ذكور وإناث الفئران المعاملة بالكوكاكولا مقارنة بالمجموعة الضابطة. كذلك نقص معنوى فى تركيز الهيموجلوبين وعدد كرات الدم الحمراء والبيضاء والصفائح الدموية مع زيادات معنوية فى الخلايا اللمفاوية وحيدة الخلية فى مجموعة الفئران التي أعطيت المشروب الغازى عنها فى المجموعة الضابطة. كما أظهرت النتائج انخفاض معنوى فى مستوى الكالسيوم فى الدم فى المجموعة المعاملة بالكوكاكولا فى كلا من الذكور والإناث مقارنة بالمجموعة الضابطة. وكذلك زيادة غير معنوية فى مستوى هرمون الغدة الدرقية ثلاثى اليود فى ذكور وإناث الفئران المعاملة بالكوكاكولا. وتستنتج هذه الدراسة أن استهلاك المشروبات الغازية عالية الفركتوز يمكن أن يؤدى إلى مشاكل صحية كثيرة.

لذلك يجب الابتعاد عن هذه المشروبات والعودة إلى المشروبات الطبيعية.