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BIOLOGICAL STUDIES ON SOME SOFT DRINKS

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ABSTRACT: *The aim of the study was to determine bone mineral density changes caused by consumption of cola, orange and soda soft drinks and the associated factors. Twenty Sprague-Dawley rats were divided into four groups. Groups 1 fed on basal diet as control and the other groups were fed basal diet and received 4ml soft drink /day / rat for 6 weeks. The bone mineral density of the rats was measured using Dual Energy X-ray Absorptiometry at the end of 6 weeks. The blood values and weights of the animals were also determined. The kidneys were removed for histopathological examination. The weight gain was higher in the groups consuming cola drinks than the control group and the other group rats (P ≤ 0.05). No significant change was detected in the blood calcium levels. There was a significant decrease in the bone mineral density of test groups when compared to the control groups (P ≤ 0.05). While the examination of the kidneys revealed general glomerular congestion and intertubular bleeding. So, it could be concluded that the decrease in bone mineral density might be related to the renal damage caused by soft drinks especially cola in addition to other related factors.*

Key words: *Soft drinks, Health effect, Bone minerals measurement , Biochemical blood parameters, Histopathological Examination.*

INTRODUCTION

Soft drinks are clearly a part of our culture and their consumption has risen steadily for more than 50 years (Paeratakul *et al.,* 2002). With the advent of modern life, the consumption of all kinds of drinks increased, but the consumption of cola drinks shows a much more markedly increase than any other drink (Bawa, 2005). Cola drinks may carry a health risk as they contain phosphoric acid (upper limit is 700 mg/l according to the Turkish Food Codex (1997), used as an acid regulator. Cola also contains caffeine as a stimulant and it has a very low pH value. With a much larger number of people and especially young people consuming cola drinks as compared to other drinks, the health effects of cola drinks is no doubt an important public health matter (Kassem and Lee, 2004).

Soda drinking is a growing phenomenon affecting all age groups in our community especially the adolescents as well as soft drinks have become part of the daily diet for many school age children all over the world (Mahmood *et al.,* 2008). The consumption of soft drinks has increased considerably during the last decades (Atsushi *et al.,* 2010). Also, the carbonated soft drinks industry continued to grow steadily as the nineteenth century progressed (David and Philip, 2006). Among them, the cola-based preparations are possibly the refreshments with the largest sales worldwide (Atsushi *et al.,* 2010). For example, the consumption of soft drinks in the United States is popular, as demonstrated by their total revenue of approximately 70.1 $ billion per year as stated by Fletcher *et al.,* (2010). Mahmood *et al.,* (2008) explained that the most common cause that leads to increase consumption of soft drinks was taste preferences.

According to Margaret *et al.,* (2009) who indicated that soft drink commonly refers to carbonated sodas that could have a caloric or non caloric sweetener. It is usually those soft drinks with sweetening such as sugar or High Fructose Corn Syrup (HFCS). The main ingredients of soft drinks product are water, sugar or other sweetener, acid, sometimes fruit juice, flavours and emulsions, colours, caffeine, preservatives, antioxidants and of course carbon dioxide as

indicated by Lucena *et al.,* (2005) ; Malik *et al.,* (2006) ; David and Philip (2006) and Margaret *et al.,* (2009) . Both Vermunt *et al.,* (2003) and Bray *et al.,* (2004) agreed with Elfhag *et al.,* (2007) who indicated that soft drinks contain calories in a fluid form that do not give satiety, and are of a low nutritional value.

Other types of soft drinks have appeared recently, these types of soft drinks known as low calories soft drinks. Global sales of non caloric carbonated soft drinks indicate that these have been a major sales growth market since 2002 as found by Levy and Tapsell (2007) and Margaret *et al.,* (2009). It is favored by some people who want to avoid the extra calories as stated by Elfhag *et al.,* (2007).

During the previous years, important concerns have been raised about the effects of soft drinks on human health as indicated by Whiting *et al.,* (2004); Tucker *et al.,* (2006); Vartanian *et al.,* (2007) ; Libuda *et al.,* (2008); Mahmood *et al.,* (2008) and Atsushi *et al.,* (2010) stated that soft drinks consumption reflect an unhealthy lifestyle. Also, Dhingra *et al.,* (2007) found that high consumption of soft drinks may be a marker for overall poor diet. Because soft drinks may displace other nutritious foods such as milk and fresh fruit and nutrients such as fiber as confirmed by Harnack *et al.,* (1999) ; Cullen and Zakeri (2004) and Vartanian *et al.,* (2007). In addition to, individuals with greater intake of soft drinks also have a dietary pattern characterized by greater intake of calories and saturated and trans fats, lower consumption of fiber and dairy products as found by Hu *et al.,* (2003); Rampersaud *et al.,* (2003); Pereira *et al.,* (2005) and Dhingra *et al.,* (2007).

Several studies showed that the excessive consumption of soft drinks leads to harmful health effects. For example, Gaby (2005) who agree with Dhingra *et al.,* (2007) explained that recent evidence suggests an association between high intake of sugar-sweetened soft drinks and the risk of obesity. More recently, it has been shown that soft drink consumption is linked to results in an increased risk of metabolic syndrome by Dhingra *et al .,* (2007). A study done by Gross *et al.,* (2004) and Margaret *et al.,* (2009) suggests an association between the increasing intake of sugar-sweetened soft drinks and the risk of diabetes. Also, several studies reported a positive association between excessive consumption of soda drinks which contain sugar-sweetened and dental caries by WHO (2003); Jensdottir *et al.,* (2004); Bawa (2005) and Holbrook *et al.,* (2006). Beside above, Prentice *et al,* (2006) who indicated that consumption of soft drinks among children was associated with impaired calcification of growing bones, lower calcium levels, and increased risk of broken bones. According to Paganini *et al.,* (2007) who agree with Saldana *et al.,* (2007), Thay has been reported that the consumption of carbonated drinks frequently at both early and advanced age leads to a decrease in bone mineral density and an increase in bone fractures (Wyshak, 2000). Authors of relevant articles state that cola drinks generally lead to a more marked decrease in bone density as compared to other carbonated drinks. Hypotheses related to this observed effect generally focus on phosphoric acid and caffeine, but it has been shown that the phosphorus/phosphoric acid and caffeine found in greater amounts in cola drinks has very little or no effect on calcium metabolism (Spencer *et al.,*1995). However, some studies reported that phosphoric acid has an effect on calcium metabolism, further that caffeine can caused an increased fractures and decreased bone mineral density, and that acid load due to consumption of cola drinks may have a negative influence on the calcium and bone metabolism (Garcia *et al.,* 2000). The generally accepted notion in light of previous studies is that the increased net acid amount entering the body together with phosphorus and caffeine disturbs the calcium balance). However, a study with drinks containing phosphoric acid but no caffeine showed no change in the calcium metabolism and further stated that drinks with cola but without caffeine have a negligible effect on calcium metabolism; it has also been stated that changes in the bones due to consumption of cola drinks may be due to

the associated decrease milk consumption (Heaney and Rafferty, 2001). Thus, there are contradictory reports on the effects of cola drinks on bone and calcium metabolism.

The chronic consumption of large amounts of cola-based soft drinks may increase the risk of chronic kidney diseases. Reports also suggest that the increasing intake of soft drinks is associated with an increase in the risk of gout as mentioned by Choi and Curhan (2008). Consumption large amount of sugar-sweetened soft drink is positively associated with other adverse health outcomes increases in blood pressure (Raben *et al.,* 2002). Reports also suggest that the increasing intake of soft drinks is associated with an increase in the risk of gout risk and of developing coronary heart disease as illustrated by Choi and Curhan (2008). Therefore, the objective of this study was to determine the effects of some soft drinks on weight, biochemical blood values, kidneys and bone mineral density of the Sprague-Dawley rats, a matter which is very important for the public health.

MATERIALS AND METHODS

Materials:

The soft drink types were obtained from a public market and were the best-selling and worldwide popular brands, they were immediately transferred to a flasks after their caps were opened to determine whether its pH changed over time, and the pH was measured at 0, 0.5, 1, 1.5, 2, 3, 4, 16 and 20 hr (Multi-Analyser F460, Qis, Oosterhout, The Netherlands).

Animals:

The laboratory animals used in the study were provided by research Institute of Ophthalmology Medical Analysis Department, Giza, Egypt.

Methods:

Phosphoric acid amounts of the soft samples were determined with Dionex Ion chromatography and IonPac Fast Anion III column, using the method provided by the manufacturer (Dionex application note: AN169, Sunnyvale, CA, USA, Smith, 1990).

Experimental Design:

At the beginning of the study, 20 Sprague-Dawley male rats, Age 10-week-old, weighting 140 ±5 g, were divided into four groups. Group 1 (the control groups) fed only on basal diet. The others received 4 ml soft drinks (cola, orange and soda soft drinks) (4 ml. /day). The animals were kept in separate cages under suitable environmental conditions (temperature 22–24°C, humidity 30–60%, 12 hr light/12 hr darkness). The rats were weighed and recorded weekly. The weight change rate of the rats was calculated at the end of the study using the following formula:

Weight change (%) = 100 × (last weight − initial weight)/initial weight

The general condition of the laboratory animals was also monitored daily. The study continued for 6 weeks. At the end of the study, general anaesthesia was administered to immobilize the rats [intraperitoneal ketamine (50 mg/kg) and dehydrobenzoperidol (2 mg/kg)], and all rats were examined with Dual Energy X-ray Absorptiometry using the GE-Lunar DPX-Pro instrument (GE Medical Systems Lunar Corporation, Madison, WI, USA, 2001) According Baim. *et al.,* (2005).

The rats were laid dorsally on the platform for measurement and images were obtained for bone mineral density and bone mineral content from the whole left femur and a 0.07-cm^2 area on the femur. The images were analysed on the computer using the Encore version 8.05 package software (GE Lunar, Madison, WI, USA, 2001).

Blood for biochemical evaluation was obtained on the same day from vena cava inferior of the rats that completed bone mineral density measurement.

Determination of Biochemical Blood Parameters:

Biochemical blood parameters were determined with instruments used for routine biochemical analyses (Modular Analytics E 170 Module, Roche Diagnostics, Indianapolis, IN, USA, for hormone

measurements; Olympus AU 2700 Autoanalyser, Hamburg, Germany, for determining other biochemical parameters, 2014).

Cholesterol was determined according to (Allain, 1974), Triglycerides (Fassati and Prencipe, 1982), VLDL cholesterol (Lee and Nieman, 1996), Uric acid was estimated according to the method described by (Fossatti and Prencipe, 1980), Thyroid hormones (free T4 and free T3) and thyrotrophin or thyroid stimulating hormone (TSH) were estimated in serum using Radioimmunoassay (RIA) as described by Patrono and Peskar (1987). The investigate minerals were determined by using Atomic Absorption Spectrophotometer (PYE Unican 929) and mercury was determined by hydride system and lead, cadmium were determined by graphite furnace system according to the method of Price (1974). Vitamins were assessment by the methods listed in A.O.A.C (1995).

Statistical Analysis:

All the obtained data were analyzed statistically with the SPSS for Windows version 11.0 software (Chicago, IL, USA). The data were presented as mean ± S.D (Snedecor and Cochran, 1972).

Histopathological Examination:

The left kidneys of all rats were removed to determine whether soft drinks caused renal damage. The removed kidneys were delivered in formalin to the laboratory to carry out the Histopathological examination. Sections taken from the samples fixed in formalin were stained with haematoxylin and eosin for examination.

Results

The pH value of the soft drinks types changed between 1.38 and 1.72. The pH value was 1.38 when the cap of the bottle was first opened, increasing to 1.72 after 1 hr and later varying between 1.44 and 1.64. The phosphoric acid content of cola samples was lower than 700 mg/l.

The weight change was calculated to be 16.03 ± 0.57% for the control group male rats and 54.18 ± 14.87 for the cola-consuming male rats. There was a statistically significant difference between the study groups and control group male rats ($P \leq 0.05$). On an average, the weight of the orange soft drink group increased (36.52 ± 6.62%) during the study, while the increase was calculated as 25.01 ± 5.66% in the soda-consuming male rats (Table 1). There was a statistically significant difference between the study groups and control group rats ($P \leq 0.05$) when compared their weight gains. It also found a statistically significant difference for weight gain ratios between the cola-consuming male and the other types of soft drinks (orange and soda) ($P \leq 0.05$). There was a significant weight increase after 6 weeks in the group given cola compared to the control group and this increase was statistically significant ($P \leq 0.05$). The weight of the animals in the control group increased 1.48 times for cola rats and 1.24 times for the orange rats. Two previous studies have not observed a weight increase due to cola consumption but both studies gave one group cola only as fluid (Amato *et al.,* 1997).

Table 1: Weight change in the rat groups during the study (%).

parameters	Group 1(control) (n = 5)	Group 2 (cola) (n = 5)	Group 3(orange) (n = 5)	Group 4(soda) (n = 5)
Weight change%	16.03 ^{d} ± 0.57	54.18 ^{a} ± 14.87	36.52 ^{b} ± 6.62	25.01 ^{c} ± 5.66

The blood samples were analyzed for parameters that might be associated with consuming soft drinks, especially for calcium, phosphorus and magnesium. Table (2) presents the results for the parameter studies carried out in all samples. The calcium and ionized calcium levels of the cola-consuming rats were found to be lower than the control group rats but this was not statistically significant ($P > 0.05$). There was a significant increase in the phosphorus and magnesium levels of the male rats consuming drinks with cola compared to the control group male rats ($P \leq 0.05$). For the other tests studied in all rats, there was a statistically significant decrease in the triiodothyronine (T_3) and thyroxine (T_4) levels and a statistically significant increase in progesterone levels in the cola-consuming male rats ($P \leq 0.05$ for all). There was also a marked increase in blood cholesterol, triglyceride and very low-density lipoprotein levels ($P \leq 0.05$). The T_3 decrease and progesterone and cholesterol increase in the cola-consuming orange rats compared to the control group were found to be statistically significant ($P \leq 0.05$). There was a marked decrease in the blood iron levels in both the cola and orange consuming rats when compared to the control group (the rate of decrease was 46.9% for cola rats group and 17.6% for orange rats group) ($P \leq 0.05$). The biochemical measurements of the study rats have shown that cola may change many biochemical parameters. Although it was focused on parameters that could be indicators for bone mineral metabolism, many parameters also changed in a statistically significant manner as compared to the control group. The changes were generally more marked in cola rats. The marked increase in the cholesterol and triglyceride levels in parallel to the increased weight in the rats consuming cola indicates that cola drinks may lead to serious cardiovascular problems, especially in males. The 31.9% decrease in the iron levels in cola group rats and the 14.9% decrease in orange rats show that it is possible for iron deficiency anemia to develop following long-term cola consumption. However, a study with detailed blood evaluation is required to clarify this matter.

Table 2. Biochemical values of the blood samples (mean ± S.D.).

Biochemical parameters	Group 1 (control) (n = 5)	Group 2 (cola) (n = 5)	Group 3 (orange) (n = 5)	Group 4 (soda) (n = 5)
Calcium (mg/dl)	11.70 [a] ± 0.90	10.99 [a] ± 0.45	10.61 [a] ± 1.02	10.70 [a] ± 0.74
Ionized calcium (mg/dl)	6.90 [a] ± 0.32	6.15 [a] ± 0.22	6.27 [a] ± 0.42	6.30 [a] ± 0.30
Phosphorus (mg/dl)	9.30 [b] ± 0.98	10.30 [a] ± 1.44	8.73 [c] ± 1.04	9.80 [b] ± 1.10
Magnesium (mg/dl)	2.90 [b] ± 0.27	3.40 [a] ± 0.23	3.33 [a] ± 0.56	3.20 [a] ± 0.41
Iron (µg/dl)	259.16 [a] ± 21.92	173.77 [d] ± 32.71 [*]	214.40 [c] ± 1.52	224.00 [b] ± ± 2.35
Uric acid (mg/dl)	0.80 [b] ± 0.17	1.31 [a] ± 0.22	1.30 [a] ± 0.64	1.30 [a] ± 0.18
Triiodothyronine (T_3)(pg/ml)	2.69 [a] ± 0.22	2.45 [a] ± 0.28	2.60 [a] ± 0.35	2.81 [a] ± 0.38
Thyroxine (T_4) (ng/dl)	3.33 [a] ± 0.48	2.53 ± 0.42 [b]	2.27 ± 0.67 [b]	2.05 [b] ± 0.58
Progesterone (ng/ml)	0.21 [d] ± 0.08	16.62 [a] ± 0.27	11.89 [b] ± 1.5	6.27 [c] ± 0.93
Cholesterol (mg/dl)	47.60 [c] ± 5.01	69.80 [a] ± 6.5	67.20 [a] ± 0.57	55.00 [b] ± 5.82
VLDL (mg/dl)	13.23 [c] ± 5.24	26.22 [a] ± 7.1	28.66 [a] ± 8.14	22.50 [b] ± 0.12
Triglyceride (mg/dl)	67.09 [c] ± 21.24	131.22 [a] ± 36.58	99.00 [b] ± 40.41	99.30 [b] ± 1.43

1-α,25-hydroxyvitamin D (ng/ml)	23.30 [b] ± 4.29	34.82 [a] ± 5.44	33.90 [a] ± 7.59	33.80 [a] ± 0.23

Calcium, ionized calcium, phosphorus, magnesium and 1-α,25-hydroxyvitamin D were measured as parameters associated with bone metabolism. Although there was a decrease in calcium and ionized calcium values in soft drink groups as observed in similar studies, there was no statistically significant change. The increased magnesium, phosphorus and uric acid values and decreased 1-α,25-hydroxyvitamin D value detected in cola group rats in the study indicate a renal disorder, and this is supported by the histopathological examination of the kidneys. The high phosphate content of the consumed drinks with cola may have led to decreased 1-α,25-hydroxyvitamin D synthesis and increased bone resorption as phosphate inhibits 1-α-hydroxylase in the kidneys (Garcia *et al.,* 2000 & Heaney and Rafferty, 2001).

Bone mineral density measurements were taken from two separate areas for each rat; the results are presented in Table 3. There was a statistically significant difference between all groups for the measurements taken from the large and small areas ($P \leq 0.01$). Although there was no significant difference for bone mineral density (both total femur and small area) measurements between the control and soda group rats, both measurements were statistically significantly different between the cola and orange consuming rats ($P \leq 0.01$). The total femur bone mineral density, bone mineral concentration and small-area bone mineral density values were significantly lower in the cola-consuming rats compared to the control group rats ($P \leq 0.05$). Both bone mineral density values were also statistically significantly lower in orange consuming rats ($P \leq 0.05$). All bone mineral density and bone mineral concentration values of the rats consuming cola were lower than those of the control group. The decrease was similar in cola and orange rats and was approximately 20%. The bone mineral density measurement results of this study are consistent with the similar study by Garcia *et al.* (2000).

Although there are many hypotheses on the decreased bone density and increased bone fractures following cola drink consumption, the main ones state that the high phosphate and caffeine content of these drinks, the very low pH causing acid load in the body and the decreased consumption of milk and drinks containing milk when cola drinks are consumed in large amounts are to blame. However, the obtained data demonstrates that cola drinks lead to renal damage, but it is difficult to say that renal damage-associated secondary changes in the mineral balance of the body leads to a decrease in the bone mineral density and an increase in fractures. Because it has not been proven that the renal changes are responsible in any way for the metabolic changes, this study need future studies on the subject.

Of course, there are a several limitations of this study like dose–effect relationship, and it would certainly be informative to add other biochemical parameters such as serum parathyroid hormone, testosterone, oestradiol, etc. This needs to be investigated in future studies.

Table 3. Bone mineral density values of the groups (normal ± S.D.).

	Group 1 (control) (n = 5)	Group 2 (cola) (n = 5)	Group 3 (orange) (n = 5)	Group 4 (soda) (n = 5)
BMD (total femur) (g/cm^2)	0.111 ± 0.009**	0.123 ± 0.021*	0.140 ± 0.004	0.148 ± 0.005
BMC (total femur) (g)	0.129 ± 0.01*	0.128 ± 0.025	0.180 ± 0.010	0.155 ± 0.035
BMD ($0.07cm^2$) (g/cm^2)	0.175 ± 0.023**	0.192 ± 0.03*	0.224 ± 0.033	0.217 ± 0.021

* Statistically significant when compared to the control group ($P \leq 0.05$);

** Statistically significant when compared to control (P ≤0.01). BMC, bone mineral concentration; BMD, bone mineral density.

Histopathological study of the kidneys revealed normal histological pattern of kidney (photo 1) and revealed extensive intertubular haemorrhage and glomerular congestion (photo 2). The general glomerular congestion and intertubular bleeding detected in the kidneys by histopathological examination indicate that cola drinks may cause renal damage. However, the glomerular congestion may be due to uric acid deposition in the glomeruli (photoes 3 and 4).

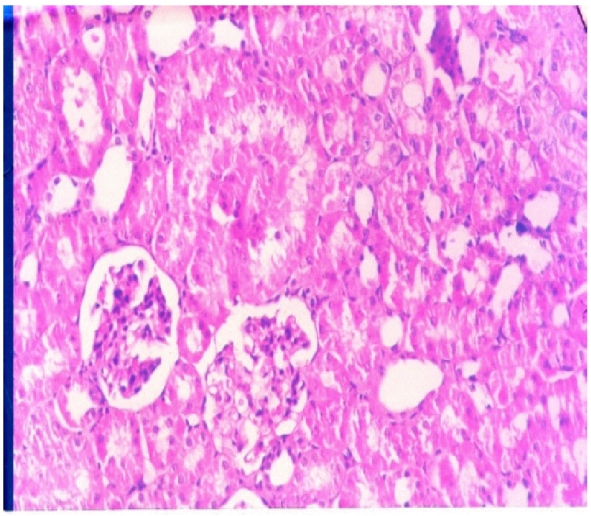

Photo (1) : Kidney of rat (Group 1) fed on basal diet showing normal histological pattern of kidney.

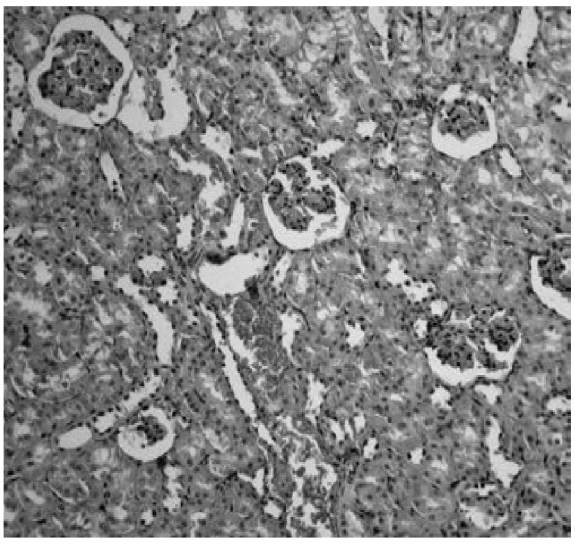

Photo 2. Kidney of rat (Group 2) showing Foci of intertubular bleeding and extensive glomerular congestion in kidney.

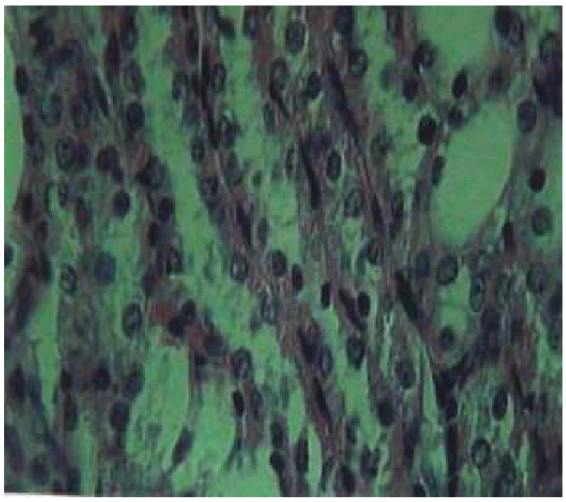

Photo (3): Kidney of rat (Group 3) showing an increase in the space between glomerulus.

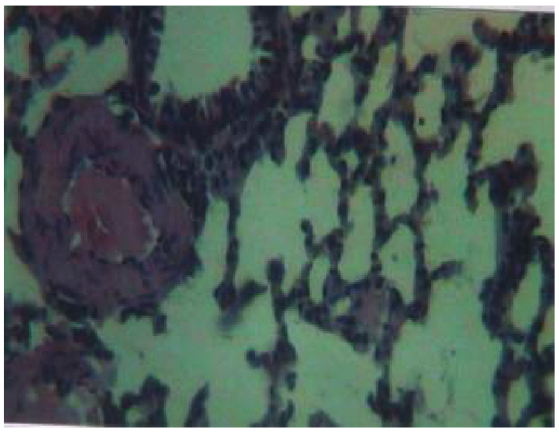

Photo (4): Kidney of rat (Group4) showing the decrease in the eosinophilic granules.

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دراسات بيولوجية على بعض أنواع المشروبات الغازية

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الملخص العربى

الهدف من هذه الدراسة هو قياس تغيرات كثافة المعادن فى العظام و التى قد تسببها إستهلاك مشروبات الكولا الغازية والبرتقال والصودا والعوامل المرتبطة بها . تم تقسيم عشرين فأر (سبراغ داولى) إلى أربع مجموعات. مجموعة (١) تتغذى على الغذاء الأساسى كمجموعة ضابطة وباقى المجموعات تتغذى على الغذاء الأساسى وتستقبل ٤ مل من المشروبات الغازية لكل فأر فى اليوم لمدة ٦ أسابيع، وقد تم قياس كثافة المعادن فى العظام للفئران بإستخدام الطاقة المزدوجة لإمتصاص الأشعة السينية فى نهاية التجربة. كما تم تقدير الاختبارات البيوكيميائية للدم وكذلك أوزان الفئران. بجانب الفحص الهستوباثولوجى. وقد اظهرت النتائج زيادة الوزن المكتسب فى المجموعة التى تستهلك مشروبات الكولا الغازية أكبر من المجموعة الضابطة ومجموعات الفئران الأخرى ($P \leq 0.05$). مع عدم وجود تغيرات معنوية فى مستويات الكالسيوم فى الدم، وإنخفاض معنوى فى كثافة المعادن فى العظام فى المجموعات المختبرة مقارنة بالمجموعة الضابطة ($P \leq 0.05$). وقد أظهر فحص الكلى وجود إحتقان كبيبى عام ونزيف فى الأنابيب الكلوية.

لذلك يمكن أن الاستنتاج أن الإنخفاض فى كثافة المعادن فى العظام قد تكون متعلقة بتلف الكلى التى تسببها المشروبات الغازية خاصة الكولا بالإضافة إلى الإرتباط بعوامل أخرى.