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# Histological and Biological Effects of Some Soft Drinks on Male Albino Rats

Nehad R. EL-Tahan[1] and Rania A. Ahmed[2]

[1]Nutrition & Food Science Dept., Faculty of Home Economics, Minoufia University, Shebin EL-Kom
[2]Zoology Department, Faculty of Science, Suez University, Suez, Egypt.

(Corresponding authors e-mail Rania.sheir@yahoo.com)

## Abstract

Soft drinks are commonly referred to non-alcoholic carbonated sodas that could have a caloric or noncaloric sweetener. Several studies showed that the excessive consumption of soft drinks leads to harmful health effects. This study aimed to determine the effects of some soft drinks on the public health by examining the histological structure of kidney, body weight, biochemical values, bone mineral density (BMD ) and bone X-ray . Twenty male rats, (weighing 140 ±5 g) , were divided into 4 groups. ; Group 1 (the control group) fed only on basal diet. ; Group 2 received cola diet soft drink ; Group 3 received orange soft drink and Group 4 received lemon soda soft drink. For histopathological examination, The kidneys were removed. The bone mineral density of the rats was assessed using Dual-energy X-ray absorptiometry (DXA). The body weight change and blood values were also determined. The histological examination of the kidneys showed general glomerular congestion, vacuolation, intertubular bleeding, tubular necrosis and hypotrophy of glomeruli . The weight gain was higher in the groups consuming diet drinks than the control group and the other group rats. There was a significant decrease in the bone mineral density of test groups when compared to the control group .

***Keywords.*** Bone , DXA , Histopathology , Kidneys , Soft drinks

## 1 Introduction

With the advent of modern life, the consumption of various kinds of drinks increases, but the consumption of soft drinks shows a much more marked increase than any other drink (Bawa , 2005). Soft drinks are commonly referred to non-alcoholic carbonated sodas that could have a caloric or noncaloric sweetener. Carbonated drinks also referred to as soda pop, soda or tonic ( Witzel and Young-Witzel ; 1998 ; and Imai *et al.* 2010) . The main ingredients of soft drinks product are water, sugar or other sweetener , flavours and emulsions, colors, sometimes fruit juice, caffeine, preservatives, antioxidants and carbon dioxide . Low calories or "Diet" soft drinks contain sweetening such as sugar or HFCS (*high-fructose corn syrup) and they* are favored by some people who want to avoid the extra calories ( Lucena *et al.* , 2005 ; David and Philip,2006 ; Malik *et al.* , 2006 and Elfhag *et al.* , 2007).

Previously , many studies showed the effects of soft drinks on human health as indicated by Imai *et al.* (2010) and Vartanian *et al.* (2007) who 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). Several studies reported a positive association between excessive consumption of soda drinks which contain sugar-sweetened and dental diseases (Bawa (2005); Jensdottir *et al.* (2004) and Cheng et al. (2009) . Both Vermunt *et al.* (2003) and Bray *et al.* (2004) agree 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.

Soft drinks may have harmful effects on health because of their phosphoric acid content (upper limit is 700 mg/l as reported by Turkish Food Codex (1997), used as an acid regulator. Also, contains caffeine as a stimulant and it has a very low pH value. With the increase of soft drinks consumers compared to other drinks, the health effects of soft drinks are no doubt an important public health matter (Kassem and Lee, 2004).

The chronic consumption of soft drinks may increase the risk of kidney diseases , gout and developing coronary heart disease as illustrated by Choi and Curhan (2008) ; and other adverse health outcomes increases in blood pressure ( Raben *et al.* ; 2002).

The aim of this study was to determine the possible effects of some soft drinks, a matter which is very important for the public health. It was investigated the effect of soft drinks on kidney, weight, biochemical blood values, and bone mineral density of the Sprague-Dawley rats.

## 2 Materials and Methods

**2.1 Animals and experiment design .**

Animals used in the study were provided by research institute of ophthalmology medical analysis department, Giza, Egypt. The study continued for 30 days. Twenty Sprague-Dawley male rats,( weighing 140 ±5 g ) , were divided into four groups .

Group 1: The control group which fed only on basal diet.

Group 2: received cola diet soft drink ( 6 ml soft drink /twice /day / rat)

Group 3: received orange soft drink ( 6 ml soft drink /twice /day / rat)

Group 4: received lemon soda soft drink ( 6 ml soft drink /twice /day / rat).

The animals were kept under suitable environmental conditions (humidity 30–60%, temperature 22–24°C, 12 hr light/12 hr darkness). The body weight change recorded at the end of the study , and was calculated using the following formula.

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

**2.2 Soft drinks**

The soft drinks were obtained from a public market and were the best-selling and worldwide popular brands. Phosphoric acid amounts of the drinks samples were measured with Dionex Ion chromatography and IonPac Fast Anion III column, using (Dionex application note. AN169, Sunnyvale, CA, USA) method. The soft drinks were transferred to a flask to determine the 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).

**2.3 Histopathological Examination .**

Rats were sacrificed and their kidneys were dissected out and fixed in 10% formal saline for 24 hours. Kidneys specimens were washed in tap water, dehydrated in ascending series of ethanol and cleared in xylene. Paraffin sections of 6 µm thicknesses were prepared by embedding specimens in paraffin wax (melting point 55-60 $^{o}$C) , then tissue blocks were cut by rotary microtome, then sections were stained with routine Haematoxylin and Eosin method (H & E) (Drury and Wallington, 1980).

**2.4 Biochemical Analysis .**

Collecting blood samples were done by using the orbital sinus technique of Sanford (1954) , then left to clot, centrifuged at 5000 r.p.m for 10 minutes. Sera were separated and stored at −20◦C.

The biochemical parameters were determined with (Modular Analytics E 170 Module, Roche Diagnostics, Indianapolis, IN, USA, for hormone measurements; Olympus AU 2700 Autoanalyser, Hamburg, Germany, for other biochemical parameters). Uric acid was estimated according to the method described by Barham and Trinder (1972). Cholesterol determined according to Allain (1974) , triglycerides (Fossati and Principe, 1982), VLDL cholesterol (Friedewald *et al.,* 1972), Thyroid hormones (free T4 and free T3) were estimated using Radioimmunoassay (RIA) (Patrono and Peskar 1987). Vitamin D was assessed by the methods listed in *A.O.A.C (1995)*. Minerals were determined by Atomic Absorption Spectrophotometer (PYE Unican 929) .

**2.5 Dual-energy X-ray absorptiometry (DXA) and - Bone mineral density (BMD)**

Rats were anesthetized by the administration of [intraperitoneal dehydrobenzoperidol (2 mg/kg) and ketamine (50 mg/kg) ], then rats were examined with dual energy X-ray absorptiometry using the GE-Lunar DPX-Pro instrument (GE Medical Systems Lunar Corporation, Madison, WI, USA). The rats were laid dorsally on the platform for measurement and images were obtained for BMD and bone mineral content from the whole left femur and a 0.07-cm$^2$ area on the femur. Images were analyzed using the Encore version 8.05 package software (GE Lunar, Madison, WI, USA).

**2.6 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).

## 3 Results

**3.1 Weight change.**

The weight change was calculated to be 16.03 ± 0.57% for the control group rats and 50.13 ± 1.87 for the drinks consuming rats. There was a statistically significant difference between the study groups and control group male rats ($P < 0.05$). 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 ($P < 0.05$) when compared their weight gains. It also found a statistically significant difference for weight gain ratios between the diet-consuming meal and the other types of soft drinks (orange and soda) ($P < 0.05$). There was a significant weight increase after 30 days in the group given compared to the control group and this increase was statistically significant ($P < 0.05$).

**3.2 Histopathological Results .**

Histopathological observation showed significant differences between the control group and the other groups which drank soft drinks (Fig. 1). Microscopic examination of kidney sections of control rats ( group 1) showed the normal structure of renal parenchyma with normal glomeruli and tubules (Fig 1a ) Sections of rat from ( group 2) showed vacuolation of epithelial lining renal tubules ( Fig.1 b ) , vacuolations of endothelial lining glomerular tufts (Fig. 1 c ) .Examination of kidney sections of group 3 rats showed congestion of renal blood vessels (Fig. 1 d ) and cystic dilatation of renal tubules (Fig. 1 e ) . Rats from group 4 showed hypotrophy in glomeruli and widening in Bowman's space, and congestion of intertubular blood capillaries associated with necrobiotic changes of epithelial lining renal tubules (Fig. 1 f) and congestion of intertubular blood capillaries (Fig. 1 g ) .

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

| parameters | Group 1( control) (n = 5) | Group 2 ( diet) (n = 5) | Group 3( orange) (n = 5) | Group 4( soda) (n = 5) |
|---|---|---|---|---|
| Weight change% | 16.03 ± 0.57 | 50.13[a] ± 1.87 | 36.52 [b] ± 6.62 | 25.01 [c] ± 5.66 |

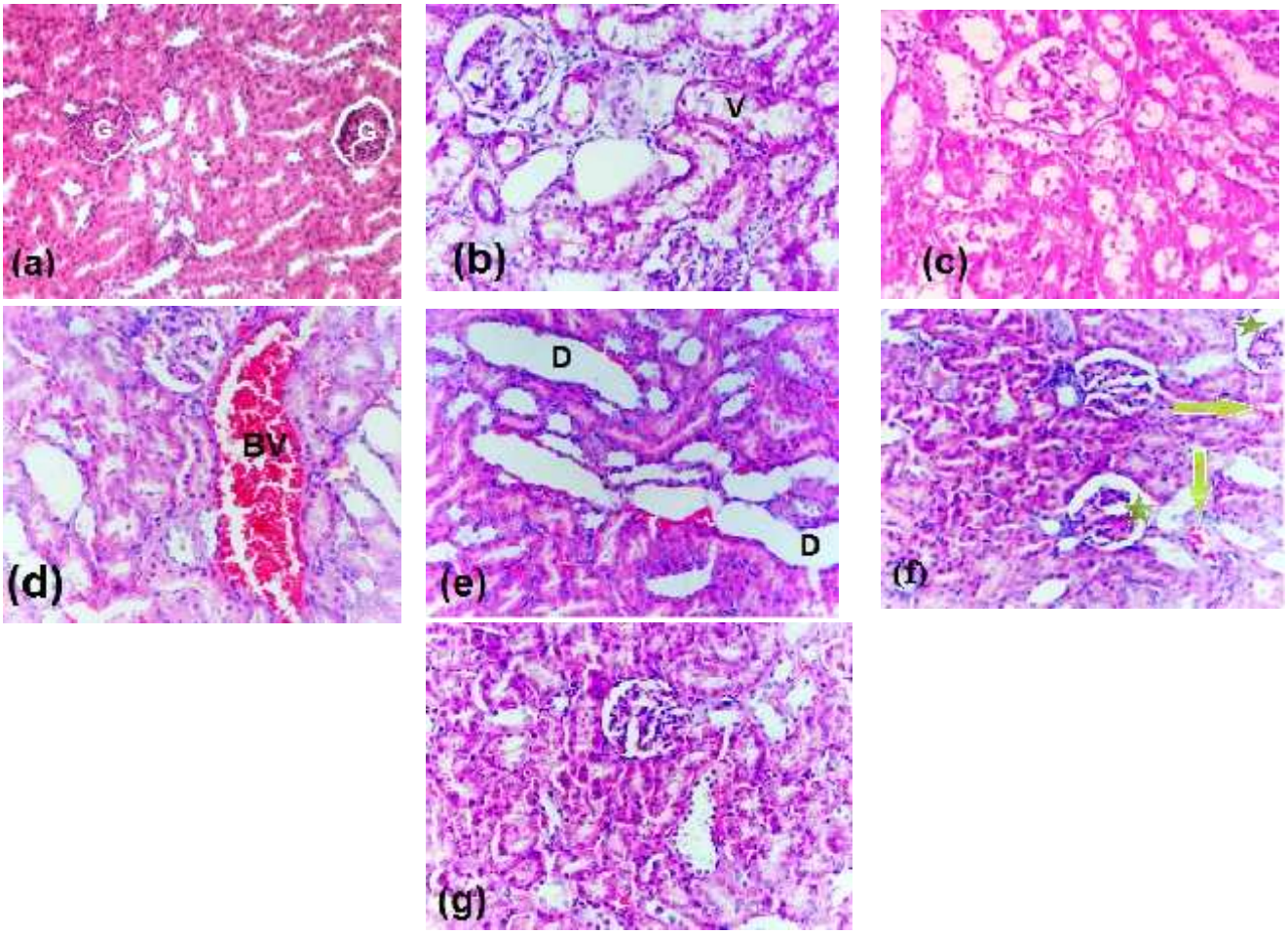


**Fig 1 .** Light microscope photomicrographs showing the histopathological alterations induced by different soft drinks in the kidney tissues of different experimental groups **(H&E stain)**

**(a)** Kidney section of a rat from control group showing normal histology of renal parenchyma with normal glomeruli (G) and tubules (×200). - **(b)** Kidney of a rat from group 2 showing vacuolation of epithelial lining renal tubules (V) (x 200). - **(c)** Kidney of rat group 2 showing vacuolations of endothelial lining glomerular tufts and epithelial lining renal tubules (×200). - **(d)** Kidney of group 3 rat showing dilatation and congestion of renal blood vessels (BV ) (X 200). **(e)** Kidney of group 3 rat showing cystic dilatation of renal tubules (D) (X 200). - **(f)** Kidney of rat from group 4 showing hypotrophy in glomeruli and widening in Bowman's space (star) , and congestion of intertubular blood capillaries (arrow) associated with necrobiotic changes of epithelial lining renal tubules (x200). - **(g)** Kidney of rat from group 4 showing congestion of intertubular blood capillaries (×200).

**3.3 Biochemical Values.**

Soft drinks intake was found to cause biochemical alterations. **Table (2)** presents the results of the parameter studies carried out in all samples. The calcium and ionized calcium levels of the 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 soft drinks when compared to the control group male rats ($P < 0.05$). 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-consuming male rats ($P < 0.05$ for all). There was also a marked increase in blood cholesterol, triglyceride and very low-density lipoprotein levels ($P < 0.05$). The $T_3$ decrease and progesterone and cholesterol increase in the diet-consuming orange rats compared to the control group were found to be statistically significant ($P < 0.05$). There was a marked decrease in the blood iron levels in both the cola diet and orange consuming rats when compared to the control group (the rate of decrease was 46.9% for diet group and 17.6% for orange rats group) ($P < 0.05$). There were also a significant increase of both 1-α,25-hydroxyvitamin D (ng/ml) and uric acid in all soft drink groups when compared with the control group .

**3.4 Bone mineral density (BMD)**

Bones were taken from two separate areas for each rat. BMD measurements showed a statistically significant difference between all groups for the measurements taken from both large and small areas ($P < 0.01$). Although there was no significant difference for BMD measurements between the control and soda group, both measurements were statistically significantly different between the diet and orange consuming rats ($P < 0.01$). The total femur BMD , BMC and small-area bone mineral density values were significantly lower in the soft drinks rats compared to the control group rats ($P < 0.05$). All BMD values were also statistically significantly lower in orange consuming rats ($P < 0.05$) (Table 3).

**3.5 Bone X-Ray Results.**

Plain X- ray on bones of upper and lower extremities AP of control negative group revealed normal bone density and no fracture line detected (Fig 2 a ) . Meanwhile, plain x-ray on bones of upper and lower extremities AP diet cola soft drink (group 2) , and lemon soft drink (group 4 ) revealed normal bone density, no fracture line detected and no periosteial reactions (Fig 2 b and Fig 2 d ) . While Plain x-ray on bones of upper and lower extremities AP of rats from orange soft drink group revealed decreased bone density and fracture shaft detected at the upper left one (Fig 2 c - arrow ).

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

| **Biochemical parameters** | **Group 1( control) (n = 5)** | **Group 2 (diet) (n = 5)** | **Group 3( orange) (n = 5)** | **Group 4(**lemon**) (n = 5)** |
|---|---|---|---|---|
| Calcium (mg/dl) | 11.70 [a] ± 0.90 | 10.93[a] ± 0.15 | 10.61 [a] ± 1.02 | 10.70 [a] ± 0.74 |
| Ionized calcium (mg/dl) | 6.90 [a] ± 0.32 | 6.55 [a] ± 0.02 | 6.27 [a] ± 0.42 | 6.30 [a] ± 0.30 |
| Phosphorus (mg/dl) | 9.30 [b] ± 0.98 | 10.80 [a] ± 1.84 | 8.73 [c] ± 1.04 | 9.80 [b] ± 1.10 |
| Magnesium (mg/dl) | 2.90 [b] ± 0.27 | 3.60 [a] ± 0.03 | 3.33 [a] ± 0.56 | 3.20 [a] ± 0.41 |
| Iron (µg/dl) | 259.16 [a] ± 21.92 | 183.77[d] ± 12.71[*] | 214.40 [c] ± 1.52 | 224.00 [b] ± ± 2.35 |
| Uric acid (mg/dl) | 0.80 [b]± 0.17 | 1.41 [a] ± 0.02 | 1.30 [a]± 0.64 | 1.30 [a]± 0.18 |
| Triiodothyronine ($T_3$) (pg/ml) | 2.69 [a] ± 0.22 | 2.55 [a] ± 0.08 | 2.60 [a] ± 0.35 | 2.81 [a] ± 0.38 |
| Thyroxine ($T_4$) (ng/dl) | 3.33 [a] ± 0.48 | 2.63 ± 0.22 [b] | 2.27 ± 0.67 [b] | 2.05 [b] ± 0.58 |
| Progesterone (ng/ml) | 0.21 [d]± 0.08 | 18.72 [a] ± 0.27 | 11.89 [b] ± 1.5 | 6.27 [c] ± 0.93 |
| Cholesterol (mg/dl) | 47.60 [c] ± 5.01 | 71.80 [a] ± 4.5 | 67.20 [a] ± 0.57 | 55.00 [b] ± 5.82 |
| VLDL (mg/dl) | 13.23 [c] ± 5.24 | 31.22 [a] ± 7.1 | 28.66 [a] ± 8.14 | 22.50 [b] ± 0.12 |
| Triglyceride (mg/dl) | 67.09 [c] ± 21.24 | 151.22 [a] ± 6.58 | 99.00 [b] ± 40.41 | 99.30 [b] ± 1.43 |
| 1-α,25-hydroxy vitamin D (ng/ml) | 23.30 [b] ± 4.29 | 34.82 [a] ± 2.44 | 33.90 [a] ± 7.59 | 33.80 [a] ± 0.23 |

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

| **BMD values** | **Group 1 (control) (n = 5)** | **Group 2 ( diet) (n = 5)** | **Group 3( orange) (n = 5)** | **Group 4**(lemon) **(n = 5)** |
|---|---|---|---|---|
| BMD (total femur) (g/cm$^2$) | 0.111 ± 0.009** | 0.113 ± 0.011* | 0.140 ± 0.004 | 0.148 ± 0.005 |
| BMC (total femur) (g) | 0.129 ± 0.01* | 0.118 ± 0.015 | 0.180 ± 0.010 | 0.155 ± 0.035 |
| BMD (0.07 cm$^2$) (g/cm$^2$) | 0.175 ± 0.023** | 0.182 ± 0.02* | 0.224 ± 0.033 | 0.217 ± 0.021 |

*Statistically significant when compared to the control group (P < 0.05);
**Statistically significant when compared to control (P < 0.01).
"BMC, bone mineral concentration ; BMD, bone mineral density."

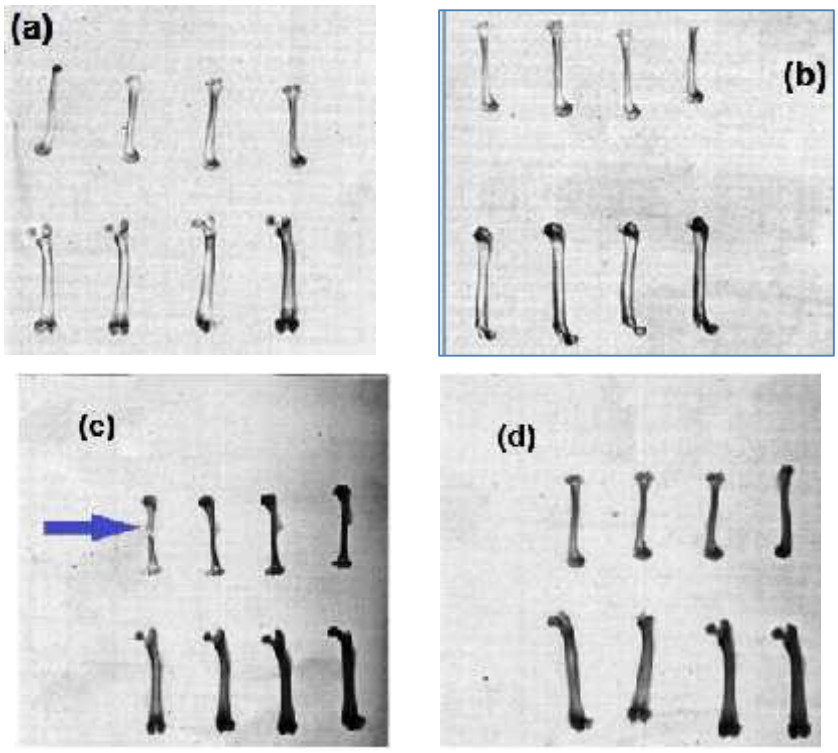


Fig 2 . Plain X- ray on bones of upper and lower extremities of rats groups ( AP view)
(a) control group - (b) cola diet soft drink group - (c) orange soft drink group (d) lemon soft drink group

## 4 Discussion

The consumption of soft drinks has a considerable increase during the last decades and their industry continued to grow steadily( David and Philip, 2006 and Imai *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).

Soft drinks were found to cause many harmful effects. Such as, detrimental effects of chronic cola consumption, enamel softening, ( Jensdottir et al. 2004 and Jensdottir et al. 2006 ) , bone demineralization (Ogur et al. 2007 and Tucker 2009) , hypokalemic myopathy (Matsunami and Imai 1994 ; Packer 2009 and Tsimihodimos et al. 2009 ). development of metabolic syndrome and diabetes mellitus (Acheson 2005 ; Assy et al 2008 ; Shoham et al. 2008 ), and chronic kidney diseases (Paganini-Hill et al 2007 and Saldana et al. 2007 ) , reproductive problems such as preterm delivery, and spontaneous abortion (Parazzini et al. 1998 ; Signorello and McLaughlin 2004 ; Bech et al. 2005 and Matijasevich et al. 2005).

The current study showed a statistically significant difference between the soft drinks groups and control group in body weight. The strongest effect on BWG showed by diet cola soft drink group unexpectedly because they contain less energy compared to other types, but it also agrees with some studies that the rising consumption of diet soft drinks provides a rising intake of aspartame which can contribute to weight gain and obesity ( Yang ; 2010 ).

Several studies also proved that the excessive consumption of soft drinks cause weight gain and obesity. For example, Gaby (2005) and Dhingra *et al*. (2007) who explained the association between high intake of sugar-sweetened soft drinks and the risk of obesity. Dhingra *et al.* (2007) found that soft drink consumption is linked to results in an increased risk of metabolic syndrome.

In the present work, there was a significant alteration in the histological structure of the kidney of all groups when compared with the control group. This is confirmed with Fahim et al. (2015) , who studied the histological effects of carbonated drinks on rat kidney and found that soft drinks caused glomerular congestion, tubular necrosis and inflammatory cell infiltration . Also, the results obtained in this experiment are in consonance with the work carried out by Enaibe et al. ( 2007 a ). They reported that administration of camphor resulted in glomerulonephritis , glomerular lobulation , mild edema, and congestion of blood cell in the kidney of rabbits.

A study by Adjene et al. (2010 ) , also supported the current results. The consumption of soft drink for 30 days caused distortion and disruption of the renal cortex cyto-architecture diffuse glomerulonephritis and congestion. According to another study by Enaibe et al. (2007 b ) , when damiana (Tunera diffusa) caused reduced size and number of renal corpuscles, necrosis in kidney histology and distortion of renal structures in Wistar rats, it.

The biochemical measurements of the presented study showed significant differences in biochemical parameters of soft drink rat groups when compared with the control group, which confirms with many studies that found marked biochemical effects of feeding soft drinks (Raj et al. , 2009 ) . The marked increase in the cholesterol , VLDL and triglyceride levels in parallel to the increased weight in the rats consuming indicates that soft drinks may lead to serious cardiovascular problems (Choi and Curhan ,2008). Soft drinks also have a dietary pattern characterized by higher intake of trans and saturated fats and calories , lower consumption of fiber and dairy products as found by Rampersaud *et al.* (2003); Pereira *et al*. (2005) ; Malik et al (2006 ) ; and Dhingra *et al*. (2007). The decrease in the iron levels in soft drink groups may induce iron deficiency anemia by soft drinks long-term consumption. However, a study with detailed blood evaluation is required to clarify this matter.

The present study measured calcium, ionized calcium, magnesium, 1-α,25-hydroxyvitamin D and phosphorus, 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 study by Amato et al (1998), there was no significant difference change. The increased uric acid , magnesium , phosphorus values and decreased 1-α,25-hydroxyvitamin D value detected in rats groups in this study indicate a renal disorder, which is matched with the histopathological alterations of the kidneys. Increased phosphate content of the soft drinks may have resulted in decreased 1-α,25-hydroxyvitamin D formation and increased bone resorption as phosphate inhibits 1-α-hydroxylase in the kidneys (Garcia-Contreras -Contreras et al., 2000 & Heaney and Rafferty, 2001).

The presented results showed that all bone mineral density and bone mineral concentration values of the rats consuming were lower than those of the control group which consistent with a similar study by Garcia-Contreras et al. (2000). Prentice (2007) indicated that consumption of soft drinks among children caused impaired calcification of growing bones decreased calcium levels, and increased the risk of broken bones. Also , Wyshak (2000) reported that the consumption of soft drinks at both young and old peoples leads to a decrease in BMD and an increase in bone fractures .

Many researchers state that soft drinks may cause marked decrease in bone density compared to other drinks. Some studies suggested that because of the high phosphoric acid and caffeine content in soft drinks, but it has been shown that the phosphorus/phosphoric acid and caffeine has a rare or no effect on calcium metabolism (Spencer *et al.,* 1965). On the other hand, several studies proved that phosphoric acid has an important role on calcium metabolism, moreover, caffeine found to increase fractures and decreased BMD , and that acid load due to consumption of drinks may have a negative effect on the bone and calcium metabolism (Garcia-Contreras et *al.* 2000). The most acceptable suggestion of previous studies is that the increased acid amount entering the body with phosphorus and caffeine disturbs the calcium balance. Heaney and Rafferty (2001) , studied the effect of drinks containing phosphoric acid only without caffeine and they found that there wasn't any change in the calcium metabolism and they reported that drinks with phosphoric acid only without caffeine have a marked effect on calcium metabolism; it has also been suggested that changes in the bones due to carbonated drinks intake may be due to the related decreased milk consumption. Thus, there are contradictory reports on the effects of drinks on bone and calcium metabolism.

However, our study proved that soft drinks lead to marked histopathological effects in the kidney, but it is difficult to say this renal damage induced by changes in the mineral balance of the body which led to a decrease in the BMD and an increase in fractures. Because it has not been proven that the renal changes are responsible in minerals metabolism, we need future studies on this subject. Of course, there are several limitations of this study like the duration of the experiment, gender of experimental animals, and it would certainly be informative to add other biochemical parameters such as serum parathyroid hormone, sex hormones , oestradiol, etc. Also, it would certainly be informative to study the effect of soft drinks consumption on female reproductive system and polycystic ovary syndrome (POS). This needs to be investigated in future studies which should include the possible protective role of neutral products against harmful effects induced by soft drinks consumption.

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