

## Effect of Carbonated Drinks on Biochemical Parameters of Rat Serum

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**Abstract:** In the recent years carbonated drinks have become frequent in diets of children, teenagers and adults. Globally there is a growing concern in the medical and scientific communities about the harmful effects associated with carbonated drinks, mainly demineralization of bones. The current study was designed to investigate the effect of carbonated drinks effect on bones demineralization. For this purpose twenty rats were sub-divided into two groups: control group, subjected to tap water (Group 1): and group supplied with carbonated drinks (Group 2) for a period of 90 days. At termination of study after 3 months, blood samples of rats were examined for their serum mineral (calcium and phosphorus) contents, glucose, lipid profiling, serum creatinine, alanine transaminase, bilirubin contents and alkaline phosphatase contents. Moreover their body weights were also monitored during the course of study. Rats consuming carbonated drinks had increased level of calcium ( $9.05 \text{ mg dL}^{-1}$ ) in blood as compared to the non-drinkers ( $7.46 \text{ mg/dL}$ ). There was also significant difference in phosphorus, blood sugar, cholesterol, high density lipoprotein, *triglycerides*, alkaline phosphatase and body *weight of rats*. There was non-significant difference in low density lipoprotein, creatinine, alanine transaminase and bilirubin. It can be concluded from current study that carbonated drinks are harmful as it de-mineralize the bones may lead to osteoporosis and risk of obesity in later life.

**Keywords:** Soft drinks, sweetened beverages, body weight, body minerals, phosphorus, glucose, osteoporosis, obesity.

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## 1. Introduction

In maintenance of bone health and metabolism nutrients including fats, proteins and sugars are crucially important (Tian and Yu, 2017). Availability of sweeteners and carbonated drinks has increased due to modern industrial development and considered as important components of our diets (Azad and Gupta, 2016; Guthrie and Morton, 2000; Mesana et al., 2018; Tappy and Le, 2010)

Personals utilizing ample quantity of soft drinks in their daily routine are more prone to health problems

as compared to others (Azagba et al., 2014; Martin-Calvo et al., 2014; Sarfaraz et al., 2017). Carbonated beverages comprises of phosphoric acid resulting in depletion of calcium content of bones (Vanishree et al., 2012). Consumption of carbonated beverages on regular intervals is known as one of the main reason in bone deterioration eventually causing osteoporotic conditions. Osteoporosis is a common disease of bones leading to enhanced bone frailty causing increased chances of skeletal failure *i.e.* fracture (Mahdi et al., 2015; WHO, 2003).

Adolescence is an important time in human growth cycle as an escalated amount of mineral

accumulation occurs in this specific time period (Bailey et al., 2000; Golden, 2018; Loomba-Albrecht and Styne, 2009; Loud, 2018). Generally it is observed that excessive utilization of carbonated drinks occur as an individual enters (Perez-Lopez et al., 2010; Whiting et al., 2001). Carbonated drinks cause calcium to leach down from bones and causes irritation in the stomach. An anti-acid is released by stomach in order to cure this irritation. This anti-acid released is actually calcium that is actually coming from the bones. After depletion of reservoirs in serum for calcium further calcium is drawn out from bones to minimize the irritation in stomach if not then brain and muscular functions are impaired (Hawkes, 2010).

Excessive consumption of carbonated drinks is associated with fewer intakes of numerous minerals and vitamins (Jacobson, 2005). These carbonated drinks possess empty calories which results in health related discrepancies like obesity, hypertension, diabetes and strokes (Bentley et al., 2018; U.S. Surgeon General, 2001). Weight gain associated with utilization of excessive carbonated drinks causes onset of type-2 diabetes in teenagers (CDC, 2004). High doses of caffeine in carbonated energy drinks could cause restlessness, hypertension, excessive urination, irritability, anxiety, sleeplessness, high blood pressure, and gastrointestinal problems (Rehm et al., 2008; Spruijt-Metz et al., 2010).

Purposely, this study was designed to investigate the effect of carbonated beverages on serum mineral content in rats. Furthermore, other biochemical parameters like serum glucose, serum creatinine and bilirubin were determined to determine toxic effect of soft drinks on various vital organs.

## 2. Materials and Methods

### 2.1 Sample Collection

Twenty albino male rats were procured from National Institute of Health (NIH) Islamabad and were brought to animal modeling room of National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad (U.A.F.). Rats were kept under controlled parameters like temperature ( $25 \pm 1^\circ\text{C}$ ) and relative humidity (45 to 55%) under 12-h light: 12-h dark cycle. The rats were randomly divided into two groups each comprising of five rats. The rats were subjected with normal feed. One group of rats was provided with drinking water (tap water and normal routine rat feed) and the other group was given carbonated drinks (carbonated drinks and normal routine rat feed) for 92 days. The

period of three months was considered sufficient to measure calcium loss in albino rats. After three months, blood sample were taken to test the calcium, phosphorus, sugar, lipid profile, creatinine, alanine transaminase, alkaline phosphatase and bilirubin. Their body weights were also measured

Body weight of rats was measured. Their blood samples were gathered at the end of study in order to examine serum mineral (calcium & phosphorus) contents, glucose, lipid profiling, serum creatinine, alanine transaminase, bilirubin contents and alkaline phosphatase contents. Bones (femur bone) of rats were taken for dimensional measurements (length and width).

### 2.2. Physical Parameter

#### 2.2.1. Body Weight

Body weight of each rat was measured by weighing balance (Mettler Toledo™, model; ME-104) on weekly basis.

#### 2.2.2. Bone Tests

The femur bone of each rat was collected. The bones were de-fleshed with care. Post wrapping of bones in saline soaked gauze they were stored at  $-20^\circ\text{C}$  till further analysis. Subsequently, each bone was weighed using digital weighing balance (Mettler Toledo™, model; ME-104). Moreover, length and width of bones were measured using a vernier caliper (Bel-Art Products, Pequannock, NJ).

### 2.3. Serum Biochemistry

Serum glucose content was analyzed at the end of three months study. The blood was obtained by tail vein puncture of rats. Serum concentration of glucose was determined by glucometer (Accu-Chek Active, Model G, Roche Diagnostics, USA).

#### 2.3.1. Calcium content

For the determination of serum calcium content calculated in  $\text{mg dL}^{-1}$ , the serum samples were analyzed spectrophotometrically at 575 nm (Barnett et al., 1973).

#### 2.3.2. Phosphorus content

For the determination of serum phosphorus content calculated in  $\text{mg dL}^{-1}$ , the serum samples were analyzed spectrophotometrically at 575 nm (Gamst and Try, 1980).

**Table 1. Body weight, bone weight, length and width in controlled and soft drink administrated rats**

Physical Parameters	Control Group	Soft Drink Administrated Group
Body Weight (g)	98.21 b ( $\pm 2.44$ )	109.33a ( $\pm 3.41$ )
Bone Weight (mg)	415.00 a ( $\pm 3.01$ )	392b ( $\pm 2.29$ )
Bones Length (mm)	32.77a ( $\pm 0.32$ )	32.71b ( $\pm 0.21$ )
Bones Width (mm)	3.83a ( $\pm 0.03$ )	3.80b ( $\pm 0.04$ )

### 2.3.3. Serum creatinine

Creatinine ( $\text{mg dL}^{-1}$ ) was analyzed by using Jaffe reaction method (Young, 1995).

### 2.3.4. Serum bilirubin

Bilirubin ( $\text{mg dL}^{-1}$ ) in blood serum of rats was analyzed using Jendrassik-Grof method (Guder and Zawta, 2001). It was measured by using Beckman Synchron LX20 method (Friedman et al., 1980).

### 2.3.5. Serum ALP

ALP in blood serum of rats was analyzed using 'Optimised standard method' according to recommendations of German Clinical Chemistry Association (Henderson and Donald, 2001).

### 2.3.6. Serum cholesterol level

Cholesterol levels were calculated in  $\text{mg dL}^{-1}$  of collected sera of rats and were measured by CHOD-PAP method as mentioned by Stockbridge et al. (1989).

### 2.3.7. Serum HDL content

The high density lipoproteins (HDL) ( $\text{mg dL}^{-1}$ ) were assessed by HDL Cholesterol Precipitant method (Assmann, 1979).

### 2.3.7. Serum LDL content

Serum low density lipoproteins (LDL- $\text{mg dL}^{-1}$ ) was calculated by following the protocol adopted by McNamara et al., (1990).

### 2.7.8. Serum Triglycerides

The triglycerides ( $\text{mg dL}^{-1}$ ) in the collected sera of rats were determined by liquid triglycerides (GPO-PAP) method as described by Ferreira et al. (2015).

## 2.3. Statistical Analysis

Interpretation of collected data was carried out by performing statistical analysis. CRD (completely randomized design) was subjected to assess the collected data. Means of treatment were compared by Duncan's new multiple range test (Steel et al., 1997).

## 3. Results and Discussion

### 3.1. Body Weight

Weights of rats were increased with daily consumption of carbonated drinks (Table 1). Controlled group had means  $98.21 \pm 2.44$  g which were increased significantly to  $109.33 \pm 3.41$  g in soft drink administrated group. These results show that carbonated drinks contribute to weight gain and can lead to obesity and other health problems due to obesity. Bocarsly et al., (2010) examined both short and long term effects of carbonated drinks on body weight of rats, body fat, and circulating triglycerides in rats. Over the course of 6 or 7 months, rats gained significantly more body weight than control group accompanied by an increase in adipose fat and elevated circulating triglyceride levels. Similar conclusions were reported by other researchers also (Bently et al., 2018; Meyers et al., 2017; Vorland et al., 2017).

### 3.2. Bone Tests

#### 3.2.1. Bone Weight

Controlled group had means  $415 \pm 3.01$  mg which were reduced significantly to  $392 \pm 2.29$  mg in soft drink administrated group (Table 1). Weight of femur bone decreased with regular consumption of carbonated drinks. There was significant effect of drinking different sugar sweetened beverages on bone mass and strength of rats (Figlewicz et al., 2009), studied separately in both female (Tsanzi et al., 2008) and male (Bass et al., 2013) rats also. The outcomes revealed difference in mass content of bone mass of rats drinking the sugar sweetened beverages despite their having the lowest food intake, but the highest beverage and caloric consumption. Rats provided the sugar sweetened beverages had reduced femur and tibia mass. Results obtained in this study were in agreement with the findings of Tsanzi et al., (2008). Weight of femur bone decreased with regular consumption of carbonated drinks. Similarly Aghili et al., (2014) also reported that carbonated soft drinks

effect bone metabolism and cause bone decalcification.

### 3.2.2. Bone Length and Width

Femur bone length of rats decreased with daily consumption of carbonated drinks. Controlled group had means  $32.77 \pm 0.32$  mm which was decreased non-significantly to  $32.71 \pm 0.21$  mm in soft drink administrated group. Similarly, results of femur bone width also had slight decrease. Rats feeding on non-carbonated drinks had means  $3.83 \pm 0.03$  mm which decreased non-significantly to  $3.80 \pm 0.04$  mm (Table 1). These results are similar to results reported by [Tsanzi et al. \(2008\)](#). Both showed non-significant reduction in femur bone length and width between rats who were provided with tap water and those who were provided with carbonated drinks.

## 3.3. Blood Biochemistry

### 3.3.1. Blood Sugar

Blood sugar of rats varied from  $86.11 \pm 1.64$  mg/dL (controlled group) to  $121.02 \pm 4.03$  mg/dL (Soft drink administrated Group) (Table 2). [Walter and Ludwig, \(2013\)](#) stated that consumption of sugar raises concentrations of insulin and triglycerides. Given these metabolic effects, the consumption of sugar sweetened drinks has been associated with an increased risk of type 2 diabetes and coronary artery disease. Blood sugar increased in rats showed highly significant results. This means that if carbonated drink is continued to be consumed regularly, the consumer is susceptible of overweight, obesity, type-2 diabetes and coronary artery disease.

### 3.3.2. Calcium Contents

Calcium level (Table 2) of controlled group had mean ( $11.50 \pm 0.16$  mg/dL) which decreased ( $9.11 \pm 0.13$  mg/dL) in soft drink administrated group showing significant result. [Fuhrman \(2012\)](#) discovered that PTH (parathyroid hormone) content elevation due to consumption of carbonated

drink. PTH performs functions to upsurge serum calcium contents by stimulating release of calcium from bone. Rats consuming carbonated drinks had more calcium in blood serum meaning that more calcium was excreted out from bones to perform physiological body functions. This will lead to low calcium in bones and can become a cause of osteoporosis.

### 3.3.3. Phosphorus

Results of phosphorus serum were seen significant in control rats ( $5.44 \pm 0.13$  mg/dL) which increased to  $7.93 \pm 0.11$  mg/dL in Soft drink administrated group (Table 2). According to [Calvo and Uribarri \(2013\)](#) elevated dietary phosphorus may be main factor giving rise to kidney failure, cardiovascular diseases and osteoporosis. Results show increase in phosphorus serum of rats which might lead to disturbed regulation of hormones giving rise to cardiovascular diseases and osteoporosis.

### 3.3.4. Creatinine

Serum creatinine level of controlled group had mean ( $0.41 \pm 0.02$  mg/dL) which decreased ( $0.38 \pm 0.01$  mg/dL) significantly in Soft drink administrated group (Table 2). No great difference was seen in serum creatinine. Elevated creatinine level signifies impaired kidney function or kidney disease ([Hall et al., 2014](#); [Levey et al., 2006](#); [Swedko et al., 2003](#)). Hence we could conclude that carbonated drinks do not cause any kidney damage.

### 3.3.5. Bilirubin

Results of bilirubin increased significantly in control rats ( $0.041 \pm 0.001$  mg/dL) which increased to  $0.072 \pm 0.002$  mg/dL in soft drink administrated group (Table 2). According to [Hawkes, 2010](#) testing for bilirubin in the blood is to see damage to your liver. Hence we conclude that carbonated drinks do not cause any liver damage as no great difference was seen in rise of serum bilirubin.

**Table 2. Mean values of Blood Sugar, Calcium, Phosphorus, Creatinine, Bilirubin, ALT and ALP in controlled and Soft drink administrated group**

Serum Biochemistry	Control Group	Soft drink administrated Group
Glucose (mg/dL)	86.11b ( $\pm 1.64$ )	121.02a ( $\pm 4.03$ )
Calcium(mg/dL)	11.50a ( $\pm 0.16$ )	9.11b ( $\pm 0.13$ )
Phosphorus (mg/dL)	5.44b ( $\pm 0.13$ )	7.93a ( $\pm 0.11$ )
Creatinine (mg/dL)	0.41a ( $\pm 0.02$ )	0.38b ( $\pm 0.01$ )
Bilirubin (mg/dL)	0.041b ( $\pm 0.001$ )	0.072a ( $\pm 0.002$ )
ALT (mg/dL)	54.80b ( $\pm 2.74$ )	69.60a ( $\pm 1.28$ )
ALP (mg/dL)	152.45b ( $\pm 4.92$ )	212.84a ( $\pm 5.32$ )

**Table 3. Mean values of Lipid profile of controlled and experimented group**

Serum Biochemistry	Control Group	Soft Drink Administrated Group
Cholesterol (mg/dL)	63.40a ( $\pm 2.95$ )	48.01b ( $\pm 2.60$ )
HDL (mg/dL)	43.40a ( $\pm 1.12$ )	37.40b ( $\pm 1.81$ )
LDL (mg/dL)	47.01a ( $\pm 2.37$ )	49.18a ( $\pm 2.85$ )
Triglycerides (mg/dL)	174.80a ( $\pm 4.27$ )	116.40b ( $\pm 4.96$ )

### 3.3.6. Alanine Aminotransferase (ALT)

Result showed increase in ALT serum by means of (54.80 $\pm$ 2.74 mg/dL) in controlled group and (69.60 $\pm$ 1.28 mg/dL) in Soft drink administrated group (Table 2). Elevated serum ALT content is a biochemical marker for liver injury (Tian et al., 2012) and its levels may assist with prognosis and guide management. Results show increase in serum ALT of rats indicating that liver functioning is disturbed (Srivastava et al., 2007).

### 3.3.7. Alkaline Phosphatase (ALP)

Result showed increase in ALP serum by means of (152.45 $\pm$ 4.92 mg/dL) in controlled group and (212.84 $\pm$ 5.32 mg/dL) in soft drink administrated group (Table 2). According to Rosalki et al., (1993) ALP in plasma originates principally from bone and liver, and measurement of these fractions is valuable for the identification of diseases of bone and of the hepatobiliary tract. ALP in rats was significant hence we conclude that consumers of carbonated drinks are at high risk of bone disease.

### 3.3.8. Cholesterol

Results were significant in both groups of rats. Controlled group had mean (63.40 $\pm$ 2.95 mg/dL) and Soft drink administrated Group had mean cholesterol level of 48.01 $\pm$ 2.60 mg/dL (Table 3). Cardiovascular diseases reported to be associated with the elevated serum cholesterol (Koyama et al., 2016; Najafi-Shoushtati et al., 2010; Nicolosi et al., 2001; Nguyen, 2018). Diet was considered as a primary tool in treating and curing cardiovascular diseases. Cholesterol serum significantly decreased in rats showing that carbonated drinks have the capacity to reduce the risk of cardiovascular diseases (Nicolosi et al., 2001).

### 3.3.9. High Density Lipoprotein (HDL)

Result showed decrease in HDL serum by means of (43.40 $\pm$ 1.12 mg/dL) in controlled group and (37.40 $\pm$ 1.81 mg/dL) in soft drink administrated group (Table 3). According to Sprecher and Stevens, (2003) low HDL serum has been proven to be a potent risk factor of cardiovascular risk. HDL of rats increased significantly. This shows that carbonated

drinks can help to raise HDL level which is beneficial for health.

### 3.3.10. Low Density Lipoprotein (LDL)

Result showed non-significant increase in LDL serum by means of (47.01 $\pm$ 2.37 mg/dL) in controlled group and 49.18 $\pm$ 2.85 mg/dL in soft drink administrated group (Table 3). Kontush et al. (2003) elaborated oxidation of LDL constitutes a key event in inflammation and atherogenesis. According to results there was no change seen in serum LDL of rats. Hence they are not considered susceptible to plaque formation in walls of arteries.

### 3.3.11. Triglycerides

Result showed decreased in triglyceride serum by means of (174.80 $\pm$ 4.27 mg/dL) in controlled group and (116.40 $\pm$ 4.96 mg/dL) in soft drink administrated group (Table 3). Rosenson et al., (2002) study stated that blood viscosity was significantly associated with triglycerides, which is a variable indicator for diabetes mellitus and may contribute to cardiovascular risk. Therefore result showed elevated level of triglycerides in blood showed more risk towards cardiovascular diseases.

## 4. Conclusion

Carbonated drinks have negative effect on calcium serum level. Excess of carbonated drinks can demineralize bones and could lead towards osteoporosis. It can also lead to over-weight and obesity if consumed regularly for long time. Serum glucose levels elevated from 86.11 $\pm$ 1.64 (control group) to 121.02 $\pm$ 4.03 mg/dL (soft drink treated group). Calcium content decreased from 11.50 $\pm$ 0.16 (control group) to 9.11 $\pm$ 0.13 mg/dL (soft drink treated group). Considering results obtained in this study, further studies are suggested to understand response of human body to excessive consumption of carbonated drinks.

**List of abbreviations:** ALP (Alkaline Phosphatase); ALT (Alanine Aminotransferase); CRD (completely randomized design); HDL (high density lipoproteins); LDL (low density lipoproteins); NIH (National Institute of Health ); PTH (parathyroid hormone)

**Conflict of Interest:** The authors have not declared any conflict of interest.

**Author Contribution:** T.N., M.I., A.A.K. designed the experiment, conducted analysis. A.L. and S.A.G. help in experimentation. T.N. and M.I. wrote the paper, S.W. and R.M.A. helped in data analysis and revision of manuscript. All authors read and approved the final draft of the manuscript.

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