IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 8, Issue 1 (Jan. – Feb. 2022), PP 36-44
www.iosrjournals.org

Effect of Soft Drink Intake in Pregnant Albino Rats and Their Fetuses

Christina Adel Zakhary[1], Mohamed Aly EL-Desouky[1], Abd EL-Wahab EL-Ghareeb[2], Heba Ali Abd EL-Rahman[2]
[1](*Biochemistry Division, Faculty of Science/ Cairo University, Egypt)*
[2](*Zoology Department, Faculty of Science/ Cairo University, Egypt)*

Abstract:
Background*: Soft drinks are currently the most popular type of beverage worldwide, with many people drinking them on a daily basis. Several studies have linked severe health consequences to the excessive consumption of soft drinks. Thus, this study aims to determine the effects of soft drink on the degree of DNA fragmentation in the placenta tissue, disturbances of biochemical parameters in pregnant albino rats, and changes in fetuses' skeletal and morphological examination.*
Materials and Methods*: Eighteen female rats, - weighing from 180g to 220 g -, were divided into three groups; (Group 1) was taken 1 ml/kg body weight of soft drink, (Group 2) was taken 2 ml/ kg body weight of soft drink, and finally; (Group 3) -control group- was taken distilled water. The period of soft drink intake was 15 days: from the 5th to the 19th day of gestation.*
Results*: The study observed a significant increase of comet percent, tail length, percent DNA in tail and tail moment of placenta of both treated groups compared with control. Significant increase of serum glucose, ALT, AST, Creatinine, Total cholesterol and triglycerides of both treated groups compared with control. Also, a significant decrease in uterus weight, fetus weight, the number of viable fetuses and implant of group 2 was noticed. The skeletal examination, observed reduction of the ossification degree of the sternum, represented curved, rudimentary and costal ribs of both treated groups, incomplete ossification of all phalanges, metacarpals and metatarsals of group 2.*
Conclusion: *The study concludes that the consumption of soft drinks during pregnancy could cause significant effects in pregnant rats and their offspring.*
Key Word*: Soft drink; Pregnant; Albino rats; Fetus.*

Date of Submission: 17-01-2022 Date of Acceptance: 31-01-2022

I. Introduction

The intake of all types of drinks increases with the advent of modern life, but the intake of cola drinks increases much more than any other drink[1]. Most soft drinks are made up of water, caffeine, sugar in the form of sucrose, phosphoric acid, and other chemicals in the form of flavors, preservatives and coloring agents[2]. Caffeine is known to increase liver enzymes, triacyl glycerol and free fatty acids and is known to produce hyperlipidemia[3]. The association between caffeine consumption and female fertility has been extensively investigated, with inconsistent results. Most studies have been retrospective, evaluating caffeine intake relative to pregnancy in women who are already pregnant. They have shown either decreased fertility or little association with fertility[4]. Soft drinks have become one of the critical harms with their increased consumption[5]. Soft drinks intake can be a sign of a bad diet because they can take the place of more healthy foods like milk and fresh fruit, as well as nutrients like fiber[6]. The consumption of soft drinks is a reflection of an unhealthy lifestyle[7, 8]. Cola drinks cause a greater decrease in bone density than other soft drinks[1]. Chronic soft drink consumption can increase blood pressure, as well as increase the risk of kidney disease, gout, and the development of coronary heart disease [9, 10]. There is a link between excessive consumption of soft drinks and dental disease[11].

Although the association between soft drinks and the public health was reported before, there is limited data on the association between soft drinks and pregnant females and fetuses. The aim of this study was to determine the possible effects of soft drinks on pregnant females and their fetuses.

II. Material And Methods

A mature females and males wistar albino rats were took from the animal house of the National Organization for Drug Control and Research (NODCAR), their weight was oscillated between (180 – 220 g). Eighteen female rats were separated into three groups, each involving six animals.

Animals and trial Groups

Group 1: Rats were treated with 1 ml/kg body weight of orally administered soda, which is the equivalent dose for humans. (Human equivalent dose 355 ml/70kg body weight therefore, dose for rat= 1ml/200g body weight according to[12].

Group 2: Rats were treated with 2 ml/kg body weight of orally administered soda which is the overdose for humans. (Human over dose 710 ml/70kg body weight therefore, dose for rat= 2ml/200g body weight)

Group 3: Control rats taken oral distilled water.

Study Location: at the animal house of Faculty of Science, Cairo University. They were housed in polyethylene cages (65×25×15 cm), with floors strewn with sawdust under light (12 h light & 12 h dark), temperature (20-23 °c) and humidity (40-50 %) conditions with the access for the needed to food and water, and all animals were placed in their housing cages for one week to adapt to experimental conditions.

Study Duration: The soft drink was given for 15 days during the 5th day to the 19th day of gestation.

Anesthetic overdose with Sodium pentobarbital (at least 100 mg/kg body weight) is a method of euthanasia. The experiment was done under guideline of Cairo University, Faculty of science Institutional Animal Care and Use Committee (IACUC) (Egypt) with approval number (CU/I/F/10/20).

Sample size: 18 Female Albino rats.

Sample size calculation: Determine the sample size depend on fetal weight.

Mating process

Male and female rats were housed for mating as the follow two females were chosen and caged with one male for a night. The following morning, vaginal smears were taken from female rats and tested. Using a pipette top, a small amount of saline was flushed into the rat's vaginal hole; then dropping into slide two drops of the resulting vaginal fluid contained cell suspension and covered with 0.1 % of methylene blue. When the smears had been dried, they were examined under a microscope using (100x) magnification. Hence, gestation day 0 was determined by the presence of sperm in the tested vaginal smears[13].

Soft drink

The used soft drink "Pepsi Cola" was obtained from a public market; highlighting the fact that this used brand is one of the most sold and popular brands around the world.

Comet assay analysis

Comet assay was developed by[14]. Generally, 50 to 100 cells were randomly selected then every sample was evaluated. Komet 5 image analysis software developed by Kinetic Imaging Ltd. (Liverpool, UK) attached to a charge coupled device (CCD) camera, was used to measure the quantitative and qualitative extent of DNA damage in the cells. Tail length (μm) is the distance of DNA migration from the center of the nuclear core's body and is used to determine the extent of DNA damage. The tail moment is the product of the tail length and the fraction of total DNA in the tail. Image analysis software was used to measure both the tail length and tail moment automatically.

Biochemical analysis

Blood samples were collected on plain tubes, allowed to be coagulated, and centrifuged at 4000 r.p.m for 10 minutes. Serums were segregated and stored at −20◦C. The biochemical parameters were determined by using colorimetric methods.

Serum glucose was estimated according to the method described by[15]. Alanine Amino Transferase (ALT) determined according to[16], Aspartate Amino Transferase (AST)[17], Creatinine[18], Total Cholesterol [19], Triglycerides [20].

Morphological examination of the uteri and fetuses

On the 20th day of pregnancy, pregnant females were sacrificed. A laparotomy was performed with exposure to the uterine horn. The uterus was removed, weighed then, opened from each female using a scissors, followed by separated fetuses. The numbers of implantation sites and live, dead and resorbed fetuses were calculated. The percentages were calculated for post-implantation loss and corrected weight gain. The live fetuses were dried on a filter paper, weighed, measured (crown-rump length) and scanned for external morphological abnormalities.

Skeletal Examination

For 10 days, the fetuses were skinned, eviscerated, then fixed in 95 % ethyl alcohol and put into a pure acetone solution to extract the fats. Alcian blue (300 mg in 100 ml 70 % ethanol) and alizarin red S (100 mg in 100 ml 95 % ethanol) stains (1 volume alcian blue, 1 volume alizarin red S, 1 volume glacial acetic acid and 17 volume 70 % ethyl alcohol) were stained for 4 days at 40º C. Then, they were then washed with tap water for a couple of hours. In the clearing process, fetuses were put in 2% aqueous KOH solution (2 g KOH in 100 ml distilled water) for 3 days, then in aqueous solution of 20% glycerin containing 1% KOH until the skeleton was clearly visible through the surrounding tissue. The cleaned fetuses were placed successively, for 7 days at each step, with 50%, 80% and 100% glycerin solution. The cartilage portions were blue, while the bones were red. Using the dissecting binocular microscope, the stained skeletons were examined to determine the skeletal malformation, such as the degree of bone ossification, the number and shape of different sections of the bone[21].

Statistical analysis

All the values were obtainable as means ± standard Deviation of the means (S.D.M). Comparison between more than two different groups was carried out using the one-way analysis of variance (ANOVA). Post hoc testing was performed for intergroup comparisons using the Duncan test, and a P value <0.05 was considered significant.

III. Result

Comet assay of DNA

The results of comet assay parameters of placenta tissue at 19th of gestation day were presented in (Table 1, Figure 1).

There was significant increase of comet percent, Tail Length, percent DNA in tail and Tail Moment in the both of treated groups (group 1 & 2) compared with the control group (group 3). In addition to, a significant increase of group 2 compared to group 1 in all this parameter.

Table no 1: Shows comet assay parameters of placenta tissue for different studied groups.

Item	Group 1	Group 2	Group 3
Comet %	$14.46\pm1.27^{a,b}$	$21.78\pm1.95^{a,b}$	8.43±0.91
Tail Length(μm)	$7.01\pm0.87^{a,b}$	$8.22\pm1.25^{a,b}$	3.26±0.66
% DNA in Tail	$9.40\pm0.59^{a,b}$	$11.94\pm0.36^{a,b}$	7.11±0.44
Tail Moment	$0.91\pm0.11^{a,b}$	$1.50\pm0.29^{a,b}$	0.26±0.04

Group 1: treated 1 ml/kg body weight, Group 2: treated 2 ml/kg body weight and Group 3: control group

Values are expressed as Mean ± SDM. The statistical differences were analyzed by ANOVA test. a= P < 0.05 compared with control and b= P < 0.05 compared with treated.

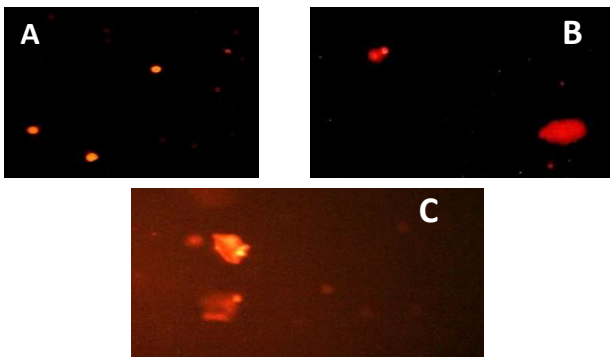

Figure 1: Shows Effect of soft drink on comet assay of different studies groups
A: Group 3 (control Group), B&C: (treated group).

Biochemical analysis

Soft drinks intake was found to cause biochemical alterations (Table 2). There was significant increase of serum glucose level, liver enzymes activity (ALT and AST), serum creatinine and lipid profile (total cholesterol level and Triglycerides) of both treated groups (group 1 & 2) compared with control group (group 3). There was a significant increase of group 2 compared to group 1 in all this parameter except glucose.

Table no2: Shows Biochemical values of the blood samples (mean ± S.D.).

Parameter	Group 1	Group 2	Group 3
Glucose (mg/dl)	190±19.9[a]	202.3±14.08[a]	142.5±7.71
ALT (IU/L)	98.66±9.30[a,b]	141.5±18.08[a,b]	67.1±8.88
AST (IU/L)	358.83±37.44[a,b]	704.16±74.93[a,b]	201.8±32.76
Creatinine (mg/dl)	1±0.11[a,b]	1.6±0.07[a,b]	0.6±0.08
Total Cholesterol (mg/dl)	84.50±4.5[a,b]	108.1±11.1[a,b]	74.1±5.1
Triglycerides (mg/dl)	690.8±99.1[a,b]	938.6±167.4[a,b]	324.1±29.43

Group 1: treated 1 ml/kg body weight, Group 2: treated 2 ml/kg body weight and Group 3: control group
Values are expressed as Mean ± SDM. The statistical differences were analyzed by ANOVA test. a= $P < 0.05$ compared with control and b= $P < 0.05$ compared with treated.

Effect of soft drink on pregnant females of albino rats

The pregnant rats of the both treated groups during gestational period (5^{th} - 19^{th} day) revealed no external symptoms of toxicity. No mortality cases were recorded, and no dead cases were round during the experimental duration. The uterus from control group revealed normal distribution of the implanted fetuses between the two horns (Figure 2) while the uterus of pregnant rats of both groups which were treated with Soda showed asymmetrical distribution of fetuses in the two uteri horn (Figure 3), Also a decrease in uterus weight of both treated groups compared to the control group was highlighted. Also there is significant ($P < 0.05$) of group 2 when compared with group 1 and group 3 (control group) (Table 3). Pregnant rats of all groups showed no post-implantation loss index.

Effect on fetus

There was a significant reduction in fetal body weight and in the number of a live fetus of group 2 with ($P< 0.05$) when compared with the group 3 (control group) and treated group 1 (Table 3). The fetuses of the control mothers have normal morphology, normal body weight, and the malformations found in fetuses of both treated groups are summarized in hematoma (red patches at different parts of the body) there was a significant elevation of hematoma after soda intake as compared with the control group (Table 3, Figure 4).

Table no 3: Shows Effect of soft drink on pregnancy outcomes and on fetal morphological abnormalities.

Parameter	Group 1	Group 2	Group 3
Uterus Weight (g)	43.67 ± 6.27[b]	23.69 ± 10.93[a,b]	50.88 ± 4.91
Fetus weight (g)	3.64±0.33[b]	2.3±0.11[a,b]	3.6±0.38
No. of viable fetuses	8.0±0.89[b]	5.6±2.5[a,b]	9.83±1.16
No. of implant	8.0±0.89[b]	5.6±2.5[a,b]	9.83±1.16
No. of dead fetus	0 (0)	0 (0)	0 (0)
No. of resorbed	0 (0)	0 (0)	0 (0)
Hematoma	2.5±0.54[a]	2.6±0.81[a]	0.3±0.8

Group 1: treated 1 ml/kg body weight, Group 2: treated 2 ml/kg body weight and Group 3: control group
Values are expressed as Mean ± SDM. The statistical differences were analyzed by ANOVA test. a= $P < 0.05$ compared with control and b= $P < 0.05$ compared with treated

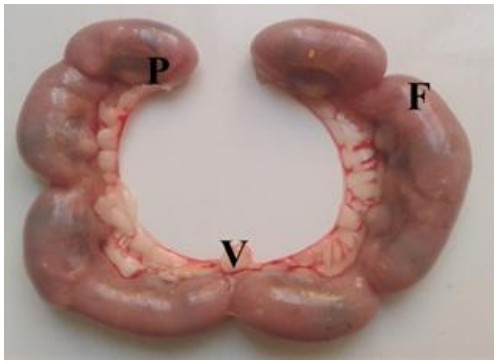

Figure 2: Shows Normal symmetrical distribution of fetuses in the two uteri horns. V= Vagina, P= placenta, F= fetuses.

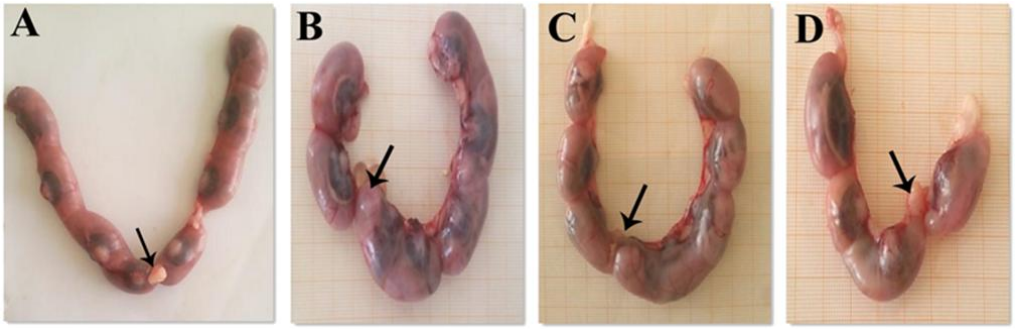

Figure 3: Shows Asymmetrical distribution of fetuses in the two uteri horn of both treated groups.

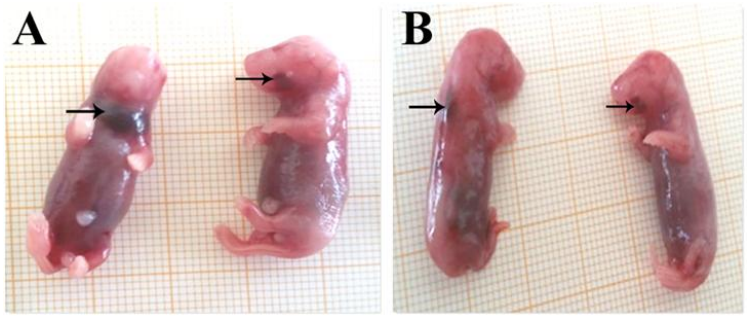

Figure 4: Shows Hematoma of the both treated groups**.**

Skeletal anomalies

Generally, the fetal skeletal system consists of two main parts; the axial and the appendicular skeleton. The axial skeleton contains the bones of skull, vertebral column, ribs and sternum. The appendicular skeleton comprises the bones of pectoral, pelvic girdles and fore, hind limbs. The vertebral column of the examined fetuses from the mother of both treated groups with soft drink showed complete ossified degree in all vertebrae. There were signs of skeletal anomalies in sternum, in fetuses of treated group of 1 ml/kg body weight (group 1) the sternum showed incomplete ossified (10/48) (20.8%) and treated group of 2 ml/kg body weight (group 2) showed lack of ossified degree (5/34) (14.7%) and incomplete ossified of sternum (21/34) (61.7%), (Figure 5). In fetuses of treated group of 1 ml/kg body weight (group 1) the ribs anomalies represented in the curved ribs (16/48) (16.6%) and rudimentary (16/48) (16.6%), and treated group of 2 ml/kg body weight (group 2) the ribs anomalies represented in curved rib (26/34) (38.23%), rudimentary (8/34) (11.7%) and costal separation (4/34) (5.8%), (Figure 6).The pectoral girdle and fore limb of fetuses maternally treated with 2 ml/kg body weight of soda (group 2) showing incomplete ossification of all metacarpals (68/68) (100%), (Figure 7). The pelvic girdle and hind limb of fetuses maternally treated with 2 ml/kg body weight of soda (group 2) showing incomplete ossified degree in metatarsals (60/68) (88.2%) and lack of ossified degree in all metatarsals (4/68) (11.7%), (Figure 8).

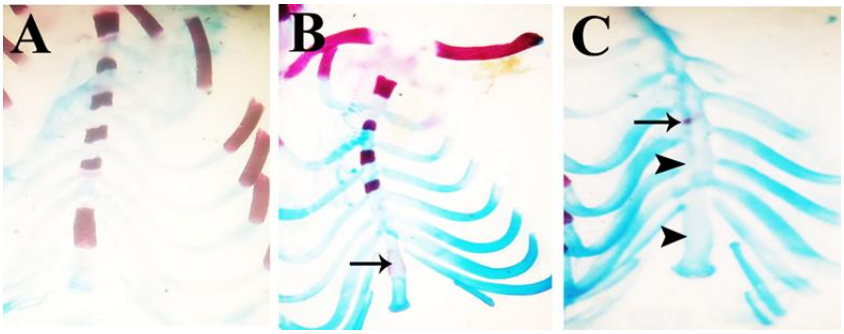

Figure 5: Shows Effect of soft drink on fetal skeletal system (sternum) at 19th gestation day
A: Group 3 (control Group), B: Group 1 (treated 1 ml/kg body weight) incomplete ossified (arrow) and C: Group 2 (treated 2 ml/kg body weight) incomplete ossified (arrow) and lack of ossified degree (head arrow).

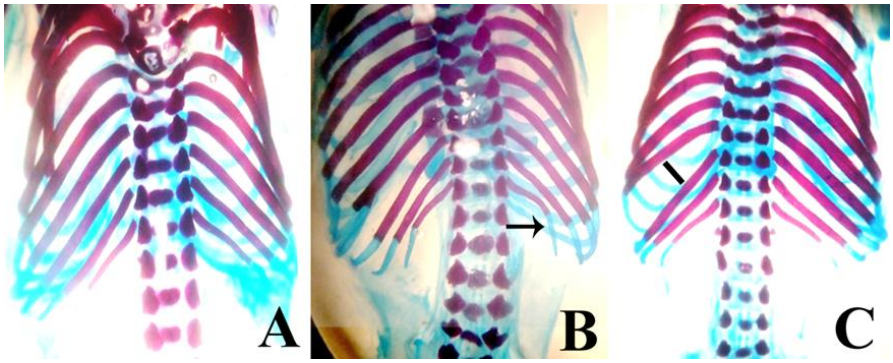

Figure 6: Shows Effect of soft drink on fetal skeletal system (ribs) at 19th gestation day
A: normal ribs, B: wavy rib (arrow) and C: costal separation (line).

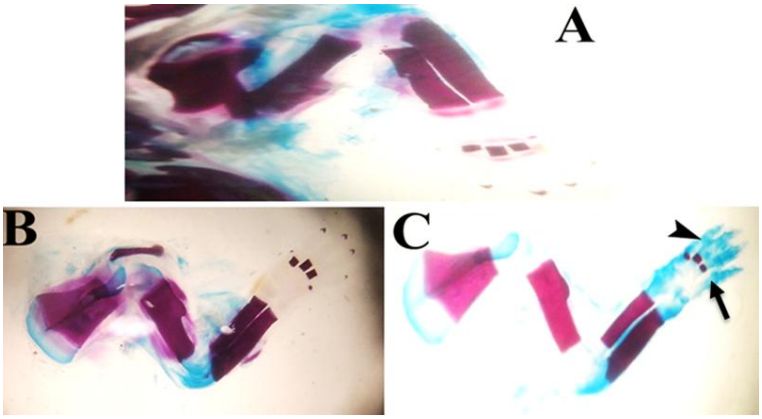

Figure 7: Shows Effect of soft drink on fetal skeletal system (pectoral girdles and forelimbs) at 19th gestation day

A: Group 3 (control Group), B: Group 1 (treated 1 ml/kg body weight) and C: Group 2 (treated 2 ml/kg body weight) incomplete ossification in all metacarpals (arrow) and phalanges (head arrow).

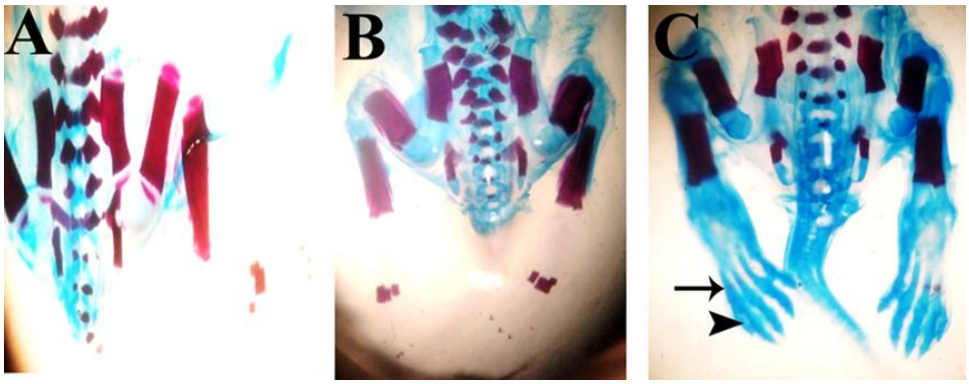

Figure 8: Shows Effect of soft drink on fetal skeletal system (pelvic girdles and hind limbs) at 19th gestation day

A: Group 3 (control Group), B: Group 1 (treated 1 ml/kg body weight) and C: Group 2 (treated 2 ml/kg body weight) incomplete and lack ossification in all metatarsals (arrow) and phalanges (head arrow).

IV. Discussion

Soft drink consumption has increased significantly in the recent decades, and their industry has continued to expand steadily[7, 22]. High level of caffeine consumption during pregnancy has been linked to miscarriage, low birth weight, growth restriction, stillbirth, and an increased risk of health problems later in life[23, 24].

Despite placenta being a transitory organ during pregnancy, it plays an important role in fetal growth. It is a multifunctional organ that serves as the liver, endocrine and exocrine glands, lung, kidney, and gut. It is the contact between the dam and the developing fetus[25].

The comet assay (also known as Single Cell Gel Electrophoresis, SCGE) is used to identify potential DNA damage after certain treatments. By monitoring the migration of DNA from immobilized nuclear chromatin, it detects DNA strand breaks and alkali-labile spots.

In the current study, DNA damage in the cell of placenta was assessed by comet assay. There was significant increase of comet percent, tail length, percent DNA in tail and tail moment of placenta of the both treated groups compared with control group.

Migration length is considered directly connected to the size of the fragment and proportional to the single-stranded breaks and alkalilabile sites[26].

This result agrees with previous results, that massive structural and numerical chromosomal abnormalities were reported together with a very large rise in the DNA fragmentation percentage in pregnant women's rats and their offspring at dose of around 50 mg/day when maternal aspartame was exposed. These findings were linked to the accumulation of Aspartame-derived methanol and metabolite formaldehyde adducts which exerted cytotoxicity through functional changes in proteins and DNA mutations leading to cell death or malignancy. Furthermore, as previously observed in rats that consume up to 1000 mg/kg/day, oxidative stress generated by aspartame metabolite can trigger nuclear damage[27, 28].

The presented study showed significant increase in biochemical parameters of both treated groups when compared with the control group. This is in line with many other studies that have discovered significant biochemical effects of soft drink consumption[3].

In the present study observed significant increase of serum glucose level, liver enzyme (ALT and AST), creatinine and lipid profile (Total cholesterol and Triglycerides) in the both treated groups as compared to control group was observed. There was a significant increase of group 2 (treated 2 ml/kg body weight) compared to group 1 (treated 1 ml/kg body weight) in all this parameter except glucose.

The glucose/fructose concentration of daily Coca-Cola is relatively high. Sugar is replaced with artificial sweeteners such as acesulfame K, aspartame, and cyclamate in Coca-Cola caffeine-free, light, and zero[29]. Chronically elevated glucose/fructose consumption may contribute to insulin resistance and, as a result, high blood sugar levels, which can lead to glucotoxicity[30].

Soft drinks can have an impact on the liver due to overconsumption, resulting in liver damage and increased ALT activity[31]. Eating fast food or drinking sugar-sweetened drinks raises the risk of elevated ALT[32]. Elevated liver enzymes and liver diseases are linked to high glycemic index carbohydrates in energy-dense unhealthy foods, as well as high amounts of sweeteners like sucrose and fructose in soft drinks and sweet snacks [33, 34]. Fructose consumption can promote de novo lipogenesis while inhibiting mitochondrial beta-oxidation of fats, resulting in increased liver fat content and elevated liver enzyme levels[35].

Increases in uric acid and creatinine levels in the blood are commonly linked to impaired kidney function [36]. Caffeine caused increases in urea, uric acid, and creatinine by inhibiting A2A adenosine receptors, leading to interstitial inflammation, increased proteinuria, and harmful changes in renal function and structure[37]. Caffeine itself is not the only contributor to the reported kidney injury, as no cases of renal injury have been reported after drinking coffee [38].

Soft drinks induce a significant increase in cholesterol and triglyceride levels in rats, indicating that they can cause severe cardiovascular issues [10]. Soft drinks also have a dietary trend that includes higher trans and saturated fat and calorie intake, as well as lower fiber and dairy product consumption [39]. High fructose intake is often associated with extremely high triglyceride levels in the bloodstream [40].

The present study showed a significant reduction of the fetuses' body weight in group 2 (treated 2 ml/kg body weight) compared to group 1 (treated 1 ml/kg body weight) and group 3 (control group), it also observed other morphological malformations such as hematoma in fetus of the two treated group compared with control group, on the other hand, the study found decreased the number of implants and viable fetuses of the treated groups compared to the control group so its effect on uterus weight. This may be due to the ability of soda to transfer across the placenta and affect the fetuses, indicating that the fetuses aren't getting enough nutrients.

Other studies have shown that excessive caffeine intake during pregnancy causes delayed conception and reduced maternal body weight gain, as well as increasing the risk of intrauterine growth retardation (IUGR) and fetus resorption [41]. Caffeine consumption before and during pregnancy has been shown to have adverse impact on reproductive function and fetal growth in rodents [42].

The present study was suitable for the study of skeletal malformation because of the high prevalence of skeletal malformation in the soft drink treated population. The types of skeletal anomalies that could be studied were the ossification degree of variety of bones of the sternum, fore, hind limbs was reduced in the animals of the both treated groups and curved and rudimentary ribs were observed. These results indicate that this may be due to the fact that decreased fetal weight has an effect on the development of the skeleton.

These results agree with other studies that, in most cases a reduction in weight is associated with a significant inhibition of skeletal growth [43]. A test compound may reduce the weight of the fetus by effects on maternal dietary intake, maternal physiology, or possibly direct effects on the fetus. Recovering body weight from offspring to control levels after delivery is common, and normal ossification is expected under these circumstances [44]. Caffeine induces delayed ossification of the fetal sternum and distal limb bones in rats at doses up to 80 mg/kg/day, but no malformations or maternal toxicity at these dose levels [45].

Wavy ribs have been classified as variations, fetal aberrations, or reversible pathologic findings rather than true malformations in rodent studies, unless they are extremely serious. Several studies have shown that wavy ribs can be reversed within a few days or weeks of birth [46].

V. Conclusion

The use of soft drinks during pregnancy could cause harmful effects to pregnant rats and their offspring as evidenced by the increase of DNA fragmentation of the placenta tissue and increase serum glucose level, liver enzyme (ALT and AST), creatinine and lipid profile (Total cholesterol and Triglycerides) and reduction of fetal weight and fetal skeletal anomalies. It was also concluded that the effects of soft drinks are dose dependent. This study is likely to be generalized to larger animals, different species of animals' models and even to human beings.

References

[1]. Ogur R, Uysal B, Ogur T, Yaman H, Oztas E, Ozdemir A, Hasde M. Evaluation of the Effect of Cola Drinks on Bone Mineral Density and Associated Factors. Basic & Clinical Pharmacology & Toxicology. 2007; 100: 334-338. [DOI: 10.1111/j.1742-7843.2007.00053.x]

[2]. Alkhedaide A, Soliman M, Salah-Eldin A, Ismail T, Alsherhiri Z, Attia H. Chronic effects of soft drink consumption on the health state of Wistar rats: A biochemical, genetic and histopathological study. Molecular Medicine Reports. 2016; 13: 5109-5117.

[3]. Raj A, Praveen KV, Varghese S, Mukkadan JK, Joseph PK. Biochemical effects of feeding soft drink and ethanol. Indian Journal of Experimental Biology. 2009; 47: 333-337.

[4]. Hatch E, Wise L, Mikkelsen E, Christensen T, Riis A, Sørensen H, Rothman K. Caffeinated Beverage and Soda Consumption and Time to Pregnancy. Epidemiology. 2012; 23: 393-401 [DOI: 10.1097/ede.0b013e31824cbaac]

[5]. Suocheng W, Huining L, Haoqin L, Luju L, Zhuandi G Coca- cola and Pepsi-cola affect ovaries and follicles development. Biomedical Research. 2016; 27(3): 6.

[6]. Dhingra R, Sullivan L, Jacques P, Wang T, Fox C, Meigs J, D'Agostino R, Gaziano J, Vasan R. Soft Drink Consumption and Risk of Developing Cardiometabolic Risk Factors and the Metabolic Syndrome in Middle-Aged Adults in the Community. Circulation .2007; 116: 480-488 [DOI: 10.1161/circulationaha.107.689935]

[7]. Imai A, Ichigo S, Takagi H, Matsunami K, Suzuki N, Yamamoto A. Effects of cola intake on fertility: a review. Health. 2010; 02: 997-1001 [DOI: 10.4236/health.2010.29147]

[8]. Vartanian L, Schwartz M, Brownell K. Effects of Soft Drink Consumption on Nutrition and Health: A Systematic Review and Meta-Analysis. American Journal of Public Health. 2007; 97: 667-675 [DOI: 10.2105/ajph.2005.083782]

[9]. Raben A, Vasilaras T, Møller A, Astrup A. Sucrose compared with artificial sweeteners: different effects on ad libitum food intake and body weight after 10 wk of supplementation in overweight subjects. The American Journal of Clinical Nutrition. 2002; 76: 721-729 [DOI: 10.1093/ajcn/76.4.721]

[10]. Choi H, Curhan G. Soft drinks, fructose consumption, and the risk of gout in men: prospective cohort study. BMJ 2008; 336: 309-312 [DOI: 10.1136/bmj.39449.819271.be]

[11]. Cheng R, Yang H, Shao M, Hu T, Zhou X. Dental erosion and severe tooth decay related to soft drinks: a case report and literature review. Journal of Zhejiang University SCIENCE B 2009; 10: 395-399 [DOI: 10.1631/jzus.b0820245]

[12]. Organization of Economic Corporation and Development (OECD). Guidelines on direct calculation of animals dose from human dose. Journal of Natural Sciences Research. 2000; 4(18): 2014.

[13]. Baker DEJ. Reproduction and breeding. In HJ Baker, JR Lindsey, SHW eisbroth. The Laboratory Rat. New York: Academic Press. 1979; 1: 153-168.

[14]. Singh N, McCoy M, Tice R, Schneider E. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research. 1988; 175 [DOI: 10.1016/0014-4827(88)90265-0]

[15]. Trinder P. Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Annals of Clinical Biochemistry: International Journal of Laboratory Medicine. 1969; 6: 24 [DOI: 10.1177/000456326900600108]

[16]. Henry JB. Clinical diagnosis and management by laboratory methods, 18th Edition. WB Saunders, Philadelphia. 1960.

[17]. Rodgerson DO, Osberg IM. Sources of error in spectrophotometric measurement of aspartate aminotransferase and alanine aminotransferase activities in serum. Clin Chem. 1974; 20: 43-50.

[18]. Vasiliades J. Reaction of alkaline sodium picrate with creatinine: 1-kinetics and mechanism of mono-creatinine picric acid complex. Clin Chem 1976; 22: 1664-1671.

[19]. Allain CC. Clin Chem. 1974; 2014: 470-475.

[20]. Fossati P, Prencipe L. Clin Chem. 1982; 28: 2077-2080.

[21]. Young B, Heath JW. Wheather's Functional Histology a Text and Colour Atlas, 4th ed. Churchill livingstone, Edinburgh. 2000.

[22]. Steen DP, Ashurst PR. Carbonated soft drinks. Formulation & Manufacture, Blackwell Publishing, UK. 2006.

[23]. Rhee J, Kim R, Kim Y, Tam M, Lai Y, Keum N. Maternal Caffeine Consumption during Pregnancy and Risk of Low Birth Weight: A Dose-Response Meta-Analysis of Observational Studies. PloS One. 2015; 10(7).

[24]. Chen LW, Wu Y, Neelakantan N, Chong M F, Pan A, van Dam RM. Maternal caffeine intake during pregnancy is associated with risk of low birth weight: a systematic review and dose-response meta-analysis. BMC Medicine. 2014; 12(174).

[25]. Sankaran S, and Kyle PM. Aetiology and pathogenesis of IUGR. Best Pract Res Clin Obstet Gynaecol 2009; 23: 765–777.

[26]. Tice, R. R. Agurell, E.; Anderson, D. Burlinson, B. Hartmann, A. and Kobayashi, H. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environmental and molecular mutagenesis. 2000; 35(3): 206-21.

[27]. Elfatah, AA, Ghaly, IS, Hanfy, SM. Cytotoxic effect of aspartame (diet sweet) on the histological and genetic structures of female albino rats and their offspring. Pak. J. Biol. Sci. 2012; 15:904-918.

[28]. Abhilash, M, Paul, MS, Varghese, MV, Nair, RH. Effect of long term intake of aspartame on antioxidant defense status in liver. Food Chem. Toxicol.2011; 49: 1203-1207.

[29]. Maes P, Monakhova YB, Kuballa T, Reusch H, Lachenmeier DW. Qualitative and Quantitative Control of Carbonated Cola Beverages Using 1H NMR Spectroscopy. Journal of Agricultural and Food Chemistry. 2012; 60: 2778-2784.

[30]. Solomon TPJ, Knudsen SH, Karstoft K, Winding K, Holst JJ, Pedersen BK. Examining the effects of hyperglycemia on pancreatic endocrine function in humans: evidence for in vivo glucotoxicity. The Journal of Clinical Endocrinology & Metabolism. 2012; 97(12): 4682-4691.

[31]. Joreh E, Awhin EP, Osademe L, Awire EI. Effect of carbonated drinks on the activities of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in serum and kidney in Rattus Novergicus. Asian Journal of Biochemistry 2012; 7(1): 59-62.

[32]. Lee EY, Choi HY, Cho H, Kim BH, Ki M. Health behavior associated with liver enzymes among obese Korean adolescents. PLoS One 2018; 13(1): 2009-2014.

[33]. Shimony MK, Schliep KC, Schisterman EF, Ahrens KA, Sjaarda LA, Rotman Y. The relationship between sugar-sweetened beverages and liver enzymes among healthy premenopausal women: a prospective cohort study. Eur J Nutr .2016; 55(2): 569–576.

[34]. Sevastianova K, Santos A, Kotronen A, Hakkarainen A, Makkonen J, Silander K. Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. Am J Clin Nutr. 2012; 96(4): 727–34.

[35]. Mirmiran P, Gaeini Z, Bahadoran Z, Azizi F. Elevated serum levels of aminotransferases in relation to unhealthy foods intake: Tehran lipid and glucose study. BMC Endocrine Disordes. 2019; 19(100): 8.

[36]. Mansy W, Alogaiel DM, Hanafi M, Zakaria E. Effects of chronic consumption of energy drinks on liver and kidney of experimental rats. Tropical Journal of Pharmaceutical Research. 2017; 16(12): 2849-2865.

[37]. Khayyat L, Essawy A, Sorour J, Al Rawi M. Impact of Some Energy Drinks on the Structure and Function of the Kidney in Wistar Albino Rats. Life Sci J 2014; 11(10): 1131-1138.

[38]. Al-Basher GI, Aljabal H, Almeer RS, Allam AA, Mahmoud AM. Perinatal exposure to energy drink induces oxidative damage in the liver, kidney and brain, and behavioral alterations in mice offspring. ClinicalKey Biomedicine & Pharmacotherapy .2018; 102: 798-811.

[39]. EL-Tahan NR, Ahmed RA. Histological and biological effects of some soft drinks on male albino rats. Journal of Bioscience and Applied Research .2015; 1(6): 335-342.

[40]. Botezelli JD, Dalia RA, Reis IM, Barbieri AR, Rezendo TM, Pelarigo JG, Codogno J, Goncalves R, Mello MA. Chronic consumption of fructose rich soft drinks alters tissue lipids of rats. Diabetology and Metabolic syndrome. 2010; 2(43): 8.

[41]. Oian J, Chen Q, Ward SM, Duan E, Zhang Y. Impacts of caffeine during pregnancy. Trends in Endocrinology & Metabolism .2020; 31(3): 218-227.

[42]. Huang J, Zhou S, Ping J, Pan X, Liang G, Xu D, Kou H, Bao C, Wang H. Role of p53-dependent placental apoptosis in the reproductive and developmental toxicities of caffeine in rodents. Clinical and Experimental Pharmacology and Physiology. 2012; 39: 357-363.

[43]. Fritz, H. Prenatal ossification in rabbits as indicative of fetal maturity. Wiley Online Library.1975; 11(3): 313–319.

[44]. DeSesso JM, Scialli AR. Bone development in laboratory mammals used in developmental toxicity studies. Birth Defects Research. 2018; 110: 1157-1187.

[45]. Collins TF, Welsch JJ, Black TN, Whitby KE, O'Donnell MWJr. Potential reversibility of skeletal effects in rats exposed in utero to caffeine. Food Chem Toxicol.1987; 25: 647–642.

[46]. Carney EW, Kimmel CA. Interpretation of Skeletal Variations for Human Risk Assessment: Delayed Ossification and Wavy Ribs. Birth Defects Research.2007; 473-496.