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Evaluation of long-term consumption of soft drinks on liver function in laboratory female rats

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ABSTRACT

Consumption of soft drinks (SDs) has increased in recent years. Soft drinks contain a collection of chemical compounds can produce multiple side effects. This work aimed to inspect the impacts of long-term consumption of SDs on liver function, antioxidant and oxidative biomarker, body weight, lipid profile and liver histological structure in female albino rats. Twenty-four female albino White rats (*Rattus norvigicus*) were randomly assigned to six groups of four animals: two control groups (groups 1-2) were fed regular pellet; Coca-Cola group (groups 3-4) were fed with standard pellet diet and given Coca Cola (2 ml) once a day; and Seven up groups (groups 5-6) were fed with standard pellet diet and given Seven up (2 ml) once a day. The treatment continued for two weeks and four months. All treatments were administered by oral gavage. Cardiac puncture technique was used for taken blood samples after 2 weeks and 3 months for measurement of liver function tests, malondialdehyde (MDA), glutathione (GSH) and lipid profile, in addition to the histological study for the liver tissue. ALAT, ASAT and ALP activity considerably elevated ($p \leq 0.05$) in animal dosed with Coca Cola and Seven up daily for 4 months. GSH significantly decrease ($p \leq 0.05$) in animal dosed with Coca Cola for 4 months. MDA was significantly increase ($p \leq 0.05$) in animal dosed with Coca Cola and Seven up for 4 months. Body weight gains significantly elevated in the treated groups for 4 months compared with the control and the other groups. Total cholesterol, TG, VLDL-C and LDL-C was increased significantly in the groups dosed with Coca Cola and Seven up for 2 weeks and 4 months compared with other groups. While the HDL-C showed a significant reduction in the groups dosed with Coca Cola and Seven up for 2 weeks and 4 months. Histopathological study revealed changes in the liver of groups treated with Cola and Seven up for 4 months represented by the appearance of a dilated and congested portal vein and ballooning degeneration of hepatocytes. In conclusions, chronic consumption of soft drinks has harmful effects on liver function and a reason for exposure to fatty liver disease.

Keywords: Soft drink, Liver function, malondialdehyde, glutathione, lipids, body weight, Histopathological study.

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1. Introduction

Through the past years, soft drinks (SDs) consumption has increased all over the world especially by children and adolescents [1]. In the Middle East, the consumption of soft drinks increased by an average of three times a day with meals [2]. Soft drink mostly contain phosphoric acid, water, sweetener such as sugar, caffeine and further preservatives, flavors, in addition to colorings [3] [2]. Increased intake of soft drinks has alarming adverse effects [1], because SDs have a high level of caffeine causing addiction, for easy and quick absorption from the intestines [4]. The SDs consumption is associated with occurrence of definite diseases, including a cardiovascular disease, obesity and diabetes mellitus [5]**.** With the increasing incidence of the diabetes mellitus, metabolic syndrome, and obesity, non-alcoholic fatty liver disease (NAFLD) as a very popular liver diseases affecting about 25 % of people around the world [6]. Disease of Non-alcoholic fatty liver does not produced through by hepatotoxic medications, extreme alcohol drinking and other established liver diseases with the fat

gathering in hepatocytes by higher than 5%, [7]. It includes a variety of liver injury that can development from steatosis, non-alcoholic steatohepatitis to hepatic fibrosis and then cirrhosis [8]. Metabolic alterations, comprising failure of lipid metabolism and insulin resistance, known as the major molecular pathogenesis of NAFLD [9]. These diseases which are like metabolic diseases such as insulin resistance, inflammation, obesity and type 2 diabetes are reflected as a liver constituent of the metabolic syndrome [10]. Consumption of fast food with soft drinks which have big quantities of saturated fat energy content, fructose, refined carbohydrates and sugar-sweetened beverages is strongly connected with increasing in body weight, obesity and newly with NAFLD [11][12].

So, this work assesses the consequence of long-term use of soft drinks on liver function, oxidative stress and antioxidants marker (MDA and GSH), blood lipids, body weight and histological changes of the liver in laboratory female rats.

2. Materials and methods

2.1 Experimental animals

Twenty-four adult albino female rats, (*Rattus norvigicus*) weighing between 190-250 g, were gotten from animal house in the Faculty of Science, University of Kufa, housing the animals were in the animal house in a standard environment include (temperature 23 - 28 °C) and controlled condition to standard laboratory nourishment with commercial food (pellets) and water provided to animals through the periods of the experiment. None of the rats had any clinically evident infections. This experimental procedure and ethics were accepted by the institutional animal care guidelines and employ committee University of Kufa.

2.2. Soft drinks doses and routs of administration

The product of soft drink used in the present study was “Coca Cola” and “Seven up”, product of Al Waha Company for Soft Drinks, Babylon, Iraq. It was purchased from a local store in Al najaf, Iraq. The animals were given a volume of 2 ml of Coca cola and Seven up each day.

2.3. Measurement of body weights

The weight of female rats was measured by using sensitive balance before the experiment and after end of the experiment.

2.4. Experimental design and blood collection

The rats were classified into six groups, each with four rats as follows: Two control groups (1-2); Coca-Cola group (groups 3-4) and Seven up (groups 5-6). Rats in groups (1-2) were fed regular pellet for two periods of time (two weeks and four months). Rats in groups (3-4) were fed with standard pellet diet and given Coca cola (2 ml) via oral gavage daily for two periods of time (two weeks and four months). While rats in groups (5-6) were fed with standard pellet diet and given Seven- up (2 ml) via oral gavage daily for two periods of time (two weeks and four months). At the end of experiment (after 2 weeks and 4 months), each animal was anaesthetized by a mix of xylazine (0.2 ml) and ketamine (0.1 ml) and they were scarified. The animals were attached to a piece of cork by using pins and then blood was drawn from the heart directly through the heart puncture to obtain adequate volume of blood (5 ml). Blood sample was put in a tube with no anticoagulant at room-temperature left for 30 minutes and used to get serum through centrifugation at 6000 rpm for 5 minutes for the biochemical tests.

2.5. Animal dissection and histological examination

The abdominal cavity of the animals was opened to eradicate the liver, and after removing the fatty tissue, the tissue samples were placed in a plastic container for the purpose of histological examination using standard procedures. The tissues were fixed by using neutral formalin (10 %) and then stained with hematoxylin - Eosin.

2.6. Biochemical analysis

2.6.1. Liver function tests

Serum Aspartate aminotransferase (ASAT/GOT) and Alanine aminotransferase (ALAT/GPT) were estimated by using UV enzymatic method KINETIC kits, and Alkaline phosphatase (ALP) were estimated by Colorimetric method KINETIC kits. All of these analyses were done according to technique provide by the LINEAR CHEMICALS/ SPAIN.

2.6.2. Antioxidant and oxidative stress biomarker

2.6.2.1. Serum Glutathione (GSH) concentration

The concentration of serum glutathione was seen by using a technique of enzyme-linked immunosorbent-assay (ELIZA), according to method provide from the Elabscience/ China/ Catalog No: E-EL- 0026 96 T.

2.6.2.2. Measurements of serum malondialdehyde (MDA) concentration

The concentration of serum malondialdehyde were evaluated by using enzyme-linked immunosorbent-assay (ELIZA) method, according to technique provide by Elabscience/ China/ Catalog No: E-EL- 0060 96 T.

2.6.3 Assessment of the lipid profile

Lipid profile assay in this study includes high density lipoprotein (HDL-C), triglycerides (TG), low density lipoprotein (LDL-C)] total cholesterol (TC), and very low density lipoprotein (VLDL-C) examined by enzymatic methods through Hitachi 7020 automatic biochemical analyzer and commercial reagents provided by (KANGXIANGMEDICAL APPliANCE, Shanghai, P.R. China).

2.7 Statistical Analysis

This study result was expressed by mean ± SE. The statistical significance was assessed by Paired Samples T test and One-way ANOVA (Analysis of Variance) through the use of the SPSS statistics software package (version 25 software) and then by using post-hoc Tukey tests. The findings are statistically significant when the $p \leq 0.05$.

3. Results

3.1. Influences of soft drinks on liver function tests

The impacts of soft drinks (Coca Cola and Seven up) on liver function of female rats are presented in Figure 1. The results indicated that ALAT was significantly elevated ($p \leq 0.05$) in animals treated with Coca Cola and seven up for 4 months and in group treated with Coca Cola for 2 weeks compared to other groups. The results also showed that the Coca Cola group treated for 4 months was significantly higher in comparison to other groups (Fig. 1A). In the same figure, the results of ASAT activity in experiment animals indicated that there was significant increased ($p \leq 0.05$) in animals treated with Coca Cola and Seven up for 4 months and in group treated with Seven up for 2 weeks in comparison to other groups (Fig. 1B). Also the results reveled that ALP was considerably elevated ($p \leq 0.05$) in the control group and Seven up for 4 months and in group treated with Seven up for 2 weeks. The results also showed that the Coca Cola group treated for 4 months was significantly higher compared to other groups (Fig. 1C).

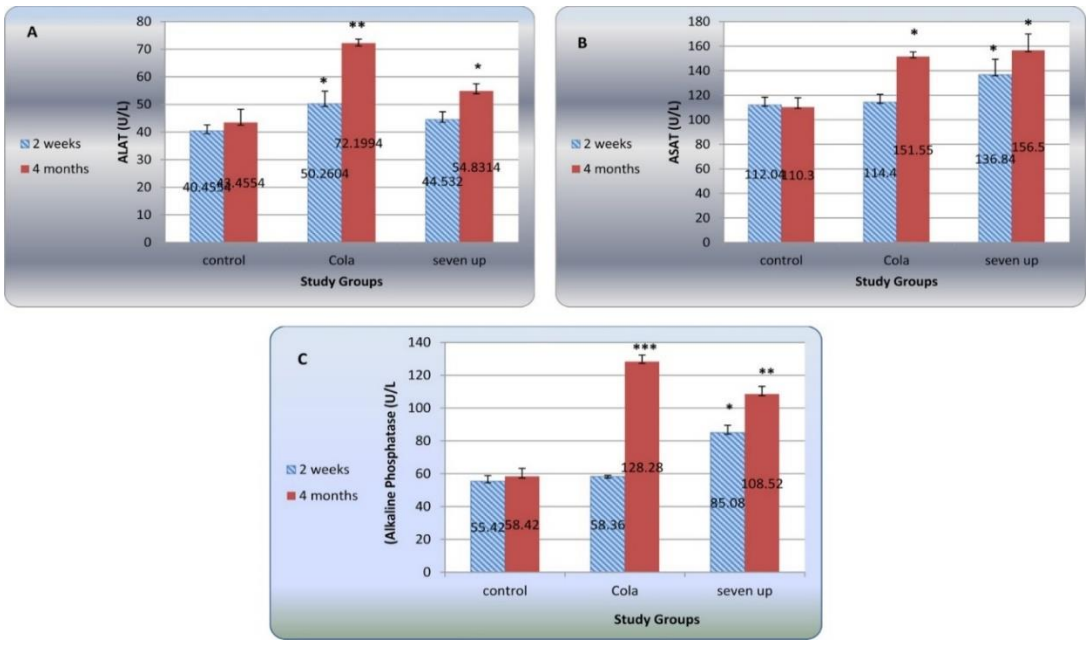

Figure 1. Comparison of alanine aminotransferase (U/L) (A), aspartate aminotransferase (U/L) (B) and alkaline phosphatase (U/L) (C) activity in serum of female rats which administrated soft drinks (2 ml) for 2 weeks and 4 months. *P < 0.05; **P < 0.01; the results are exposed as mean ± SE (n = 4)

3.2. Effect of soft drinks on antioxidant and oxidative stress biomarker

The effects of the two types of soft drink on the levels of antioxidant glutathione GSH in rats are presented in (Fig. 2A), the results indicated that GSH was significantly reduced ($p \leq 0.05$) in animal consuming Coca Cola for 4 months. Also, there was slight and non-significant decrease in GSH on animal treated with Coca Cola for 2 weeks, Seven up foe 2 weeks and 4 months. The soft drink influence on the level of oxidative stress marker MDA in rats are presented in (Fig. 2B), the results indicated that MDA was significantly increase ($p \leq 0.05$) in animal treated with Coca Cola and Seven up for 4 months, in addition there was non-significant increase in GSH on animal treated with Coca Cola and Seven up for 2 weeks.

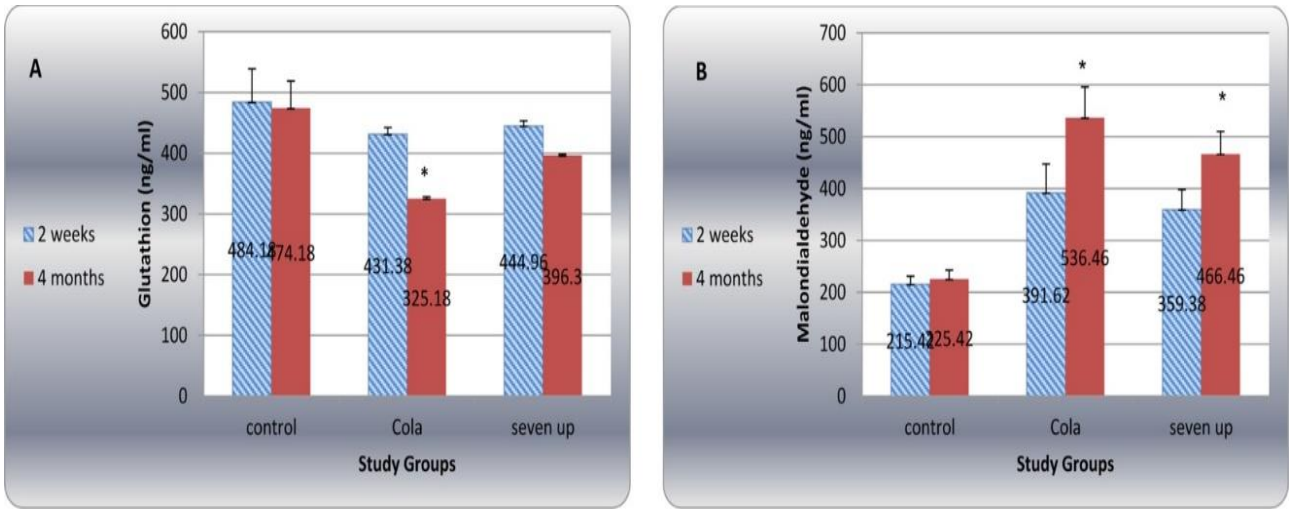

Figure 2. Glutathione (GSH) (A) and malondialdehyde (MDA) (B) level in serum of female rats which administrated soft drinks (2 ml) for 2 weeks and 4 months. **P < 0.05 ; **P < 0.01; the findings are exposed as mean ± SE (n = 4)*

3.3. Effects of soft drinks on body weight

The results in table (1) show a comparison of animal weights before and after administration soft drinks, the data exhibited considerable rise ($p<0.05$) in body weights post treatment for all groups compared with body weights pretreatment. While the results of weight gain revealed that there was a significant elevate in the treated groups for 4 months in comparison to the control and the other groups.

3.4. Effect of soft drinks on lipid profile

Serum lipids include total cholesterol, TG, LDL-C and VLDL-C were increased meaningfully in the experimental groups treated with Coca Cola and Seven up for 4 months compared with other groups. In addition, TG and VLDL-C was significantly increased in groups treated with Coca Cola and Seven up for 2 weeks. While, the HDL-C showed a significant reduce in the groups dosed with Coca Cola and Seven up for 4 months compared with other group (Table 2).

Table 1. Body weight and weight gain in female rats consuming soft drinks (2 ml) for 2 weeks and 4 months comparison with control rats

Body weight (g) / Study Group		Pre treatment Means±S.E.	Post treatment Means±S.E.	Sig.	Weight gain (g) Means±S.E.	Sig.
1 weeks	Control (N= 4)	192.30±3.462	205.00±4.549*	0.001	12.70±1.260	0.003
	Coca cola (N= 4)	197.80±1.356	222.40±2.158*	0.003	24.60±3.171	

	Seven up (N= 5)	193.60±7.159	214.60±6.438*	0.001	21.00±1.140	
4 Months	**Control (N= 4)**	198.10±5.080	217.60±4.822*	0.009	19.23±4.32	
	Coca cola (N= 4)	178.60±8.846	250.00±11.631*	0.000	71.40±15.118**	
	Seven up (N= 4)	190.20±6.506	239.80±11.297*	0.041	49.60±16.684*	

P < 0.05; **P < 0.01; the findings are exposed as mean ± SE (n = 4)

Table 2. Lipid profiles in serum of female rats which administrated soft drinks (2 ml) for 2 weeks and 4 months comparison with control rats

Lipid profile / Study Group		**Ch.(mg/dl)**	**TG (mg/dl)**	**HDL-C(mg/dl)**	**VLDL(mg/dl)**	**LDL(mg/dl)**
2 weeks	**Control (N= 4)**	83.62 ± 3.53	82.74 ± 2.19	41.68 ± 3.90	16.54 ± 0.43	25.39 ± 4.47
	Coca cola (N= 4)	79.94 ± 3.37	124.22± **0**.59*	37.36 ± 0.85	24.84± 0.11*	17.73 ± 2.64
	Seven up (N= 5)	75.99 ± 4.82	95.84 ± 5.66	41.20 ± 1.35	19.16 ± 1.13	15.62 ± 5.04
4 Months	**Control (N= 4)**	85.21 ± 3.04	84.27 ± 3.34	41.39± 5.93	17.27 ± 1.45	20.45 ± 3.23
	Coca cola (N= 4)	113.19± 4.03*	153.14± 7.01*	32.36± 0.85*	30.62± 1.40*	50.20±1.80*
	Seven up (N= 4)	110.91± 1.47*	159.42±11.61*	34.24 ± 0.52*	31.88± 2.32*	44.78±3.28*
		0.002	0.005	0.009	0.001	0.003

P < 0.05; **P < 0.01; the findings are exposed as mean ± SE (n = 4).

3.5. Effects of SDC on liver histopathology

The general architecture of the liver in females rats treated with Coca Cola and Seven up (2 ml) for 4 months were distorted with: a dilated and congested portal vein and ballooning degeneration of hepatocytes (Fig.3 E and F), While the results show: the normal histological structure of central vein with the normally arranged plate or hepatocytes in control group (Fig. 3 A and D) and in females rats treated with Coca Cola (2 ml) for 2 weeks (Fig. 3 B) and females rats treated with Seven up (2 ml) for 2 weeks (Fig. 3C).

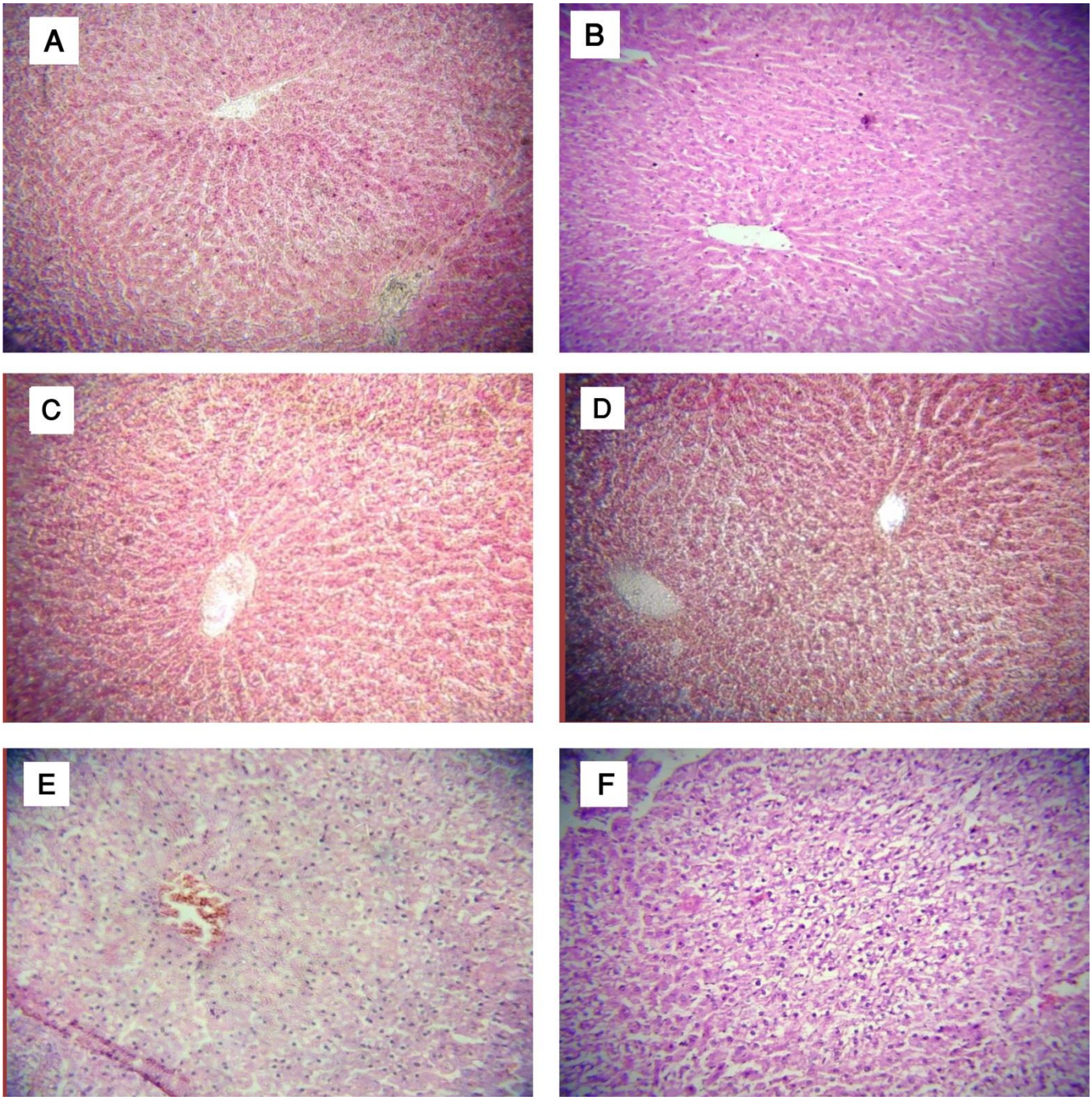

Figure 3. Photomicrograph of the liver show: the normal histological structure of central vein with the normally arranged plate or hepatocytes in control group (A and D) and in females rats treated with Cola (2 ml) for 2 weeks (B) and females rats treated with Seven up (2 ml) for 2 weeks (C). The liver in females rats treated with Cola (2 ml) for 4 months show a dilated and congested portal vein and ballooning degeneration of hepatocytes (E). Also, the liver in females rats treated with Seven up (2 ml) for 4 months show: a congested portal vein and ballooning degeneration of hepatocytes (F) (Specimens in 10% neutral formalin and stained with H and E, magnification ×40, ×10).

4. Discussion

In this current experiment study, the results revealed that the activity of the ALAT, ASAT and ALP was significantly elevated in female rats which daily dosed with Coca Cola and Seven up for 4 months. Also, daily Cola consumption for 2 weeks was associated with elevated the ALAT activity. While Seven up consumption for 2 weeks was associated with elevated the ASAT and ALAT activity. These results agreed with the previous

studies which confirmed the adverse impacts on the soft drink chronic use on liver functions. Jeroh *et al* study proved that there was a rise in the activity of liver enzymes (GOT and GPT) as a result of prolonged consumption of soft drinks which cause liver injuries, as well as the increase in the activity of the alkaline phosphatase as a bone remodeling biomarker [13]. According to Welsh *et al,* soft drinks or Sugar-sweetened beverages mainly are the producers of added sugars [14]. The caloric sweeteners in Sugar-sweetened beverages, high fructose corn syrup and sucrose seem the most regularly used fructose-containing sugars. Higher risk of fatty liver problems would be correlated with higher habitual Sugar-sweetened beverages drinking [15]. Moreover, other studies have revealed that increase likelihood of non-alcoholic fatty liver disease (NAFLD) and metabolic syndromes is connected to consuming soft drinks [16]. Soft drinks contain a big quantity of sugar and extreme energy content increasing quickly postprandial glucose levels and insulin [17]. Lebda *et al*. indicated that increase the levels of ALT representing liver inflammation connected to long- term use of soft drinks [18]. Numerous research works stated that Sugar-sweetened beverages consumption could raise the risk of hyperuricemia in humans by severe reduction of adenosine triphosphate (ATP) levels [19]; this could subsequent raise ALAT concentration. It must be well-known that the essential mechanisms for the observed relationship of sugar-sweetened beverages drinking and ALAT levels could not be related to liver fat-accumulation only, as elevated ALAT indicates liver inflammation. The data of the current study confirmed that chronic soft drinks administration caused significant decrease in glutathion (GSH) on animal dosed with Coca Cola for 4 months, also there was slight and non-significant decrease in GSH on animal dosed with Coca Cola for 2 weeks and Seven up for 2 weeks and 4 months. Also, the results confirmed that chronic soft drinks administration induced oxidative stress represented by a significant increase in oxidative stress marker malondialdehyde (MDA) on animal dosed with Coca Cola and Seven up for 4 months, also there was non-significant increase in GSH on animal dosed with Coca Cola and Seven up for 2 weeks. This result was confirmed by several studies which indicated that chronic soft drinks consumption promotes oxidative stress, modifications in metabolism and alterations in gene expression on laboratory Wistar- rats. The pathogenesis and etiology of several chronic diseases has been related with oxidative stress process which playing an important role in aging [20]. Increasing in the levels of reactive oxygen species (ROS) or free radicals is able to directly destruct lipids in the cells and stimulate peroxidation [20]. Endoplasmic reticulum, mitochondria, peroxisomes and plasma membrane are the foremost causes for ROS production inside the cells by several mechanisms, including auto-oxidation of numerous molecules including hydroquinone and catecholamines and/or enzymatic reactions [21]. Chronic soft drinks consumption promote oxidative stress, causing liver toxicity, was specified by the decrease in catalase, GSH-R and GSH-Px levels, and the elevated in MDA, along with the decline in the expression of the mRNA levels of glutathione S-transferases (GST) and superoxide dismutase (SOD) [22]. The failure of the body's antioxidant protection mechanisms to remove high concentration of reactive oxygen species could mainly cause oxidative stress in the bodies tissues [23]. Thus, degenerative diseases, such as hepatopathies is instigated by this oxidative stress [22]. The process of lipid peroxidation was established to prompt disruption of membrane integrity and function [24]. This is revealed by the rise of serum MDA concentration, which is one of the greatest frequently used biochemical markers for the process of lipid peroxidation [1]. Therefore, the elevated concentration of MDA by reason of consumption of soft drinks pointing to elevated lipid peroxidation. In addition, it is an indicator of the possible carcinogenic consequence of consuming soft drinks, as MDA is assumed to create under the condition of the stress and is greatly able to react with many molecules including DNA and proteins that cause the creation of adducts [25]. This expected possible carcinogenic consequence of soft drinks consumption is increased with the decrease of concentration of antioxidant enzymes, catalase and GSH-Px. Lebda *et al*. hypothesized that chronic intake of sweetened soft drink significantly increased changes in serum transaminases enzyme with hepatic oxidative stress such as an increased production of malondialdehyde (MDA) and decrease of glutathione peroxidase, catalase, and superoxide dismutase activities followed by oxidative hepatic injury. Several histological changes were observed in the liver such as degeneration, necrosis, infiltration, and fibrosis, mostly caused by aspartame. Lebda *et al* results propose that chronic soft drink use or aspartame made by hepatic injury is facilitated through stimulating lipid accumulation, hyperglycemia and oxidative stress along the participation of adipocytokines [18]. The results of current study confirmed that giving soft drinks for long periods of time caused a significant increase in weight gain in the rats treated with Coca Cola and Seven up for 4 months in comparison to the other groups. Also, weight gain in the rats treated with Coca Cola and Seven up for 2 weeks increased slightly. This result was explained by many other studies, which showed that sugar-sweetened beverages including soft drinks with a high-fructose corn syrup or sucrose to be sweetened, and this compound are the essential source of added sugars [14]. Luger *et al*. study have proven that excess sugar-sweetened beverages consumption was connected with weight gain [26]. One study recommends that greater drinking of sugar-sweetened beverages may be

related with fat accumulation in visceral adipose tissues and therefor increase in weight gain [27]. In addition, in a study conducted on some strains of mice, the results revealed rise in accumulating visceral fat if fed a high-fructose diet [28]. In addition, soft drinks have long contributed partly to obesity epidemic. In the latest years, many epidemiologic research work studied the relationship of long- term soft drinks drinking with weight gain. Also, in the previous two decades, obesity amongst children has significantly increased and even approached epidemic proportions [29]. The results of serum lipids include total cholesterol, TG, VLDL-C and LDL-C was increased significantly in the groups treated with Coca Cola and Seven up for 4 months compared with other groups. In addition, TG and VLDL-C was significantly increased in groups treated with Coca Cola and Seven up for 2 weeks. While the HDL-C revealed a significant decline in the animal groups treated with Coca Cola and Seven up for 4 months compared with another group. In their experiment at laboratory rats, Lebda *et al*., discussed that dietary consuming sweetened drinks with fructose corn syrup are related with the obesity and metabolic syndrome. They assumed that chronic consumption of soft drink significantly induced hyper-triacylglycerolemia and hyperglycemia , as characterized by elevated serum triacylglycerol, very low-density lipoprotein cholesterol, low-density lipoprotein and glucose, along apparent visceral- fatty- deposition, and this leads to metabolic syndromes and related with the peroxisome proliferator down-regulation being an activated receptor-γ and adiponectin and up-regulation of leptin expression. [18]. After fructose absorption from the small intestine, it moved through the portal system to the liver for metabolization to fructose-1-phosphate by the enzyme fructokinase. The enzyme aldolase cleaves fructose-1-phosphate to form glyceraldehyde-3-phosphate and glycerone phosphate, together could be additional metabolized in the pathway of glycolytic [30]. A rise in the serum triglycerides and low-density lipoprotein cholesterol levels could help improving fatty acid synthesis, augmented esterification of fatty acids and a rise in the very low-density lipoprotein [30]. Soft drink comprising about (32.6 g) of fructose can rise fructose 4 fold of the fasting serum [31]. The general architecture of the liver in female's rats treated with Coca Cola and Seven up for 4 months in this current study were distorted with: a dilated and congested portal vein and ballooning degeneration of hepatocytes, these changes are the histological features of disease of non- alcoholic fatty liver. Fatty liver Hepatic steatosis could characterize nonalcoholic fatty liver diseases [32]. Sugars, predominantly fructose are a feature of the diet that is presumed to increase nonalcoholic fatty liver disease [33]. In some randomized controlled research works discussed that there is a rise in consuming fructose related to liver fat disease [34], and others have not [35]. In other studies which conducted on mice and ducks, the results of this study proved that high fructose diets have stimulated fatty liver [36]. Such diets have furthermore caused rises in stimulation of inflammatory pathways and hepatic lipid peroxidation in the liver of laboratory rats [37]. The meta-analysis study of He *et al*, exposed that consuming soft drinks and red meat was linked to the rise in the probability of developing nonalcoholic fatty liver disease [12]. In addition, Mirmiran *et al*., study indicated that consumption of sugar- and artificially sweetened soda and red meat is linked positively to fatty liver disease [38]. There are several studies that have explained the significant plausible relationship between nonalcoholic fatty liver disease and sugar-sweetened beverage uses in persons addicted to eating sugar-sweetened beverages. The most important of them was the study of Carvalhana *et al*. showing a fructose corn syrup, as the key sweeteners in different sugar-sweetened beverages, and that the most of the research works revealed that it stimulates fatty liver disease [39]. Fructose can stimulate lipogenesis by upregulation of carbohydrate response elementary and sterol regulatory element-binding protein-1c, regulating numerous lipogenic genes [40] elevating free fatty acid in hepatic pool [41]. Furthermore, relying on 'two hits' hypothesis of nonalcoholic fatty liver disease pathophysiology as Day and James states [42] steatosis conversion to nonalcoholic fatty liver disease is connected to the factors (second hit), for instance advanced glycation end product (AGEs), lipid peroxidation, oxidative stress, inflammatory process, and resistance of insulin. Therefore, intake sugar-sweetened soft drinks possibly could be the key reason for the second hit responsible for the pathogenesis of diseases of nonalcoholic fatty liver developments [43].

5. Conclusion

The results of this study proved that the consumption of soft drinks daily for long periods was a reason for the increase in the liver enzymes activity, and this is evidence of liver function damage. Also, the consumption of soft drinks is a major cause of elevated of blood lipids and increase in the body weight. Increase the concentrations of malondialdehyde and low glutathione give evidence of the high generation of free radicals and thus the occurrence of oxidative stress. In addition, the study confirmed that continuous soft drinks consumption is a risk factor for progress of nonalcoholic fatty liver diseases.

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