Effect of Prolonged Overdose Sorbitol and Aspartame Administration on Serum Lipid Profile: Experimental Finding

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Abstract

The aim of this study was to investigate the effect of Prolonged Overdose Sorbitol and Aspartame Administration on serum Lipid Profile levels in female Rats. Blood samples were obtained from (18) adult female rats that treated with (100 mg/day) of sorbitol and (18) rats that treated with of Aspartame for 30 days, as well as (18) healthy rats that were given (Normal Saline) for 30 days as a control group. They divided into three groups as the following: Sor Group:- Included eighteen female rats that treated with (100 g/day) of sorbitol, Asp Group:- Included eighteen female rats that treated with (100 g/day) of Aspartame control Group:- Included (18) healthy rats that was given (Normal Saline) for 30 days. Results: the results in this study showed no significantly different ($p \leq 0.05$) in (TC), (TG), (HDL), (LDL), (VLDL) levels in the treated group with Asp in comparison with control group while serum (TG) and (vLDL) showed significant increase ($p \leq 0.05$) in treated group with Sor in comparison with control group, also the study reveals significant increase ($p \leq 0.05$) in serum (TG) and (vLDL) in treated group with Sor in comparison with treated group with Asp while there were no significant different ($p \leq 0.05$) in serum (TC), (HDL), levels in the group that treated with Sor in comparison with group treated with Asp and control group. In conclusion: The use of overdoses than permissible of sorbitol and aspartame for a prolonged period leads to disturbance of lipid profile levels, but it is statistically imperceptible.

Keywords: *Aspartame, Artificial Sweetener, Lipid profile, Sorbitol.*

Introduction

Artificial sweeteners: are used as sugar substitutes called ‘’zero’’ or ‘’light’’ beverages, foodstuffs, pharmaceuticals, and it is used by consumers to acquire a sweet taste without increasing caloric intake., is a low calorie option for people who should or need to limit their sugar intake, or energy control[1,2,3] are can be divided into two large groups:(a) nutritive sweeteners such as “sorbitol” which has a systematic name d-glucitol, is a 6-carbon sugar alcohol, Its sweetness equals 60 % that of sucrose [4,5]. It is widely accepted by the food and pharmaceutical industries as nutritive ingredient because of its ability to improve the taste and shelf-life of regular food. Sorbitol is partially absorbed into the body from the gastrointestinal tract and metabolized by the liver mainly as fructose and non-absorbed part is metabolized by colonic bacteria[6,7,] The initial steps in sorbitol metabolim in the liver, its uptake by liver cells and conversion to glucose is independent of insulin, but the subsequent use of glucose by the muscle and adipose tissues is influenced by insulin [8,9] this polyol can be naturally found in apples, pears, peaches, apricots and nectarines as well as in dried fruits, such as prunes, dates and raisins and in some vegetables[10,11] and (b) non-nutritive sweeteners such as “Aspartame”which has (L-aspartyl- L-phenylalanine methyl ester) also known as ‘’Nutra Sweet’’ is one of the most popular synthetic artificial sweeteners[12]**.** They are characterized by a minimum caloric value at the used doses, so they give dietetic character to foodstuffs which they are added to[13,14]**,** When aspartame is ingested it is broken down

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in the digestive tract into ordinary food components[15]. It is hydrolyzed in the intestinal lumen to aspartic acid (which makes up approximately 40% of the molecule), phenylalanine, (which makes up approximately 50% of the molecule) and methanol by proteolytic and hydrolytic enzymes[16]. Aspartame is known to have a 'clean sweet taste' and It is white crystalline powder that is colourless when dissolved . is a compound 200 times sweeter than sugar, considered as a low-calorie sweetener (4 kcal/g) and can be used as a tabletop sweetener or in frozen desserts, gelatins, beverages[17,18]. In a liquid system, it is most stable in the pH range of 3 to 5, with an optimum pH of 4.2. This Is why many diet soft drinks will decrease in sweetness over an extended period of time [16,19].

JECFA has defined an acceptable daily intake ADI) for sorbitol as "not specified," and thus, no limits are placed on its use,Otherwise, it may appear laxative effect when eaten in excess[10,11], while FDA recommended [50 g/day] as acceptable daily intake of sorbitol sweetener for humans [20]. Ingesting large amounts of sorbitol can lead to flatulence, abdominal pain, and Osmotic diarrhea as a result of intestinal malabsorption when to take a dose is greater than 50 g/day [9]. Consumption of [20–30 g/day] results in abdominal pain[21]. While the acceptable daily intake of aspartame is [40-50 mg/kg] per day, respectively, set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[22,23] the results of some experimental and epidemiological studies showed that their overdose consumption may cause some adverse health effects including obesity[24,25,26], metabolic syndrome[27,28] alteration in gut microbiota[29,30] cancer and adverse neurobehavioral effects.[31,12]

The health risks of artificial sweeteners consumption is still a highly controversial topic[3] sweeteners have allegedly been related to some effects such as overweight, metabolic disorders, migraines, type-2 diabetes, Vascular disorders, preterm delivery, kidney function disorders, liver antioxidant system, hepatotoxicity, immune system disruptions and alteration of gut microbiota activity. However, other studies, have shown association with kidney function decline and vascular risk factors[32]. The majority of the research found negligible association between the use of sugar substitutes and changes in blood lipid profiles, some RCTs found greater high density lipoprotein (HDL-Cholesterol) level among participants who consumed Aspartame compared to control who did not use sugar substitutes[33], a 'strong review' and a 'weak review' found no effect of artificial sweeteners on blood lipid profiles in adults[34,35], some identified studies, most showed no effects on lipid parameters related to the consumption of artificially sweetened beverages. A single study reported a positive association between the consumption of these beverages and an increase in TG concentrations associated with a lowering of HDL-C[36].

Epidemiological studies have shown that elevated concentrations of total cholesterol especially LDL-cholesterol in the blood are powerful risk factors for atherosclerotic cardiovascular diseases (CVD), including ischaemic heart disease and stroke[37] also for the development of other health disorders such as metabolic syndrome, type 2 diabetes mellitus and hypertension[38], Dyslipidaemia is a metabolic disturbance which stimulates insulin resistance in adipose and muscle tissues then results in free radicals [39]. which contribute to oxidative stress[40]. The formation of these free radicals and end products, and subsequent oxidative stress causes damage to endothelial tissues [41]. The dysfunction of endothelial tissue can stimulate atherosclerotic events on blood vessels, which can progress to cardiovascular diseases[40]. Dyslipidaemia becomes atherogenic when there is combined elevation of TG and (LDL-c), and decreased (HDL-c) in the blood [42].

HDL-cholesterol and LDL-cholesterol are two main groups of plasma lipoproteins that are involved in lipid metabolism and the exchange of cholesterol, cholesterol ester and triglycerides between tissues [43]. Numerous population studies have shown an inverse correlation between plasma HDL-cholesterol levels and risk of cardiovascular disease, implying that factors associated with HDL-cholesterol protect against atherosclerosis. Some of these factors appear to have antioxidant and anti-inflammatory effects which may obviate processes that initiate atherogenesis [44,45]. In Western countries, it was estimated that 45% of heart attacks were due to abnormal blood lipids. Between (2007-2017), the Global Burden of Diseases, Injuries, and Risks Factors Study (GBD) reported that the number of ischaemic heart disease deaths attributed to high LDL-C increased 20.7% [46], the majority of observational studies showed no effects on lipid profile related to artificial sweeteners. Two studies reported that replacing sugars with aspartame reduced plasma triglyceride concentrations but the data are too limited to conclude that artificial sweeteners have a beneficial effect on lipid profile [47].

Therefore, the aim of this study is to highlight the effect of continuous and prolonged administration of disallowed doses of sorbitol and aspartame on blood lipids concentrations in laboratory rats.

Animals and Method

Design of Study: Eighteen healthy adult female rats weighing (1200-1300 g) of (18-24) weeks old were used for the present study. all animals were maintained under standard laboratory conditions (12h light: 12h night cycle (LD) at 22 ± 2 Cº and relative humidity 45-55%. The animals were fed with normal laboratory diet and allowed to drink water ad libitum, All the experimental procedures conducted in the animal house of Biology Dept/College of Science/Thi-Qar University. Animals were housed in iron boxes bedded with wooden chips, during the experimental period Six animals were kept in each box and they were housed**.**

Experimental animals were divided into three groups (18 femel rats in each group) upon the following designed:

Contrl group: Control (normal) that were given (Normal Saline) for 30 days

Sor group: Rats were daily treated with(100 g/day) of sorbitol for 30 days

Asp group: Rats were daily treated with(100 g/day) of sorbitol for 30 days.

Collection of Blood Samples: Three mL of blood were drawn from each animal of experimental groups, after being anesthetized with a (diethyl ether) sniffing, dissecting it, and drawing blood directly through the heart puncture method. the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 (rpm) for 10 minutes, the serum samples were separated and stored at (-20ºC) for later measurement of biochemical parameters, unless used immediately.

Determination of Biochemical Parameters: Several considerable method were used to measure the studied parameters. It is notable that all measurements were duplicated for each sample. Serum total cholesterol (TC), triglycerides (TG) and HDL-cholesterol were estimated by enzymatic colorimetric method. The used reagents were supplied by Biolabo (France), LDL was calculated according to ‘’Friedwald’ formula. Non-HDL was measured by subtracting HDL from TC as: [LDL(mg/dl) = Total cholesterol – (HDL + VLDL)]. and VLDL concentrations were measured as follows : [VLDL(mg/dl) = serum TG/5]

Statistical Analysis: Statistical analysis was done using the software **(**Excel, 2010) the results were expressed as mean ± standard deviation (mean ± SD). One way (ANOVA-single factor) was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant (t= 2.131).

Result and Discussion

Serum Lipid Profile Concentrations: Recently, concerns have been raised about the safety of artificial sweeteners that are commonly used as substitutes for sucrose in many diet products[48]. Therefore in our study we tried to examin the effects and biochemical variations on lipid profile (serum total cholesterol, triglycerides, HDL and LDL-Cholesterol) in blood and tissues of experimental rats treated with high dose of Sorbitol and Aspatame,Data showing the effect of sweeteners in Table 1**.**

Table (1) showed the results of serum lipids and lipoproteins levels. Serum cholesterol (TC) levels were no significantly different ($p \leq 0.05$) in the treated groups with Sor and with Asp in comparison with control group.

Serum triglyceride (TG) and (vLDL)levels were significantly increase in (Sor treated groups) in comparison with (Asp treated group) and control group ($p \leq 0.05$) as shown in table (1). No significant variations were observed in the levels of TG and vLDL in the Asp treated groups when the comparison with control group.

Serum (HDL) levels shown no significant difference ($p \leq 0.05$) in Sor and Asp treated groups in comparison with control group.

Serum (LDL) level were significantly increased in (Sor treated groups) in comparison with (Asp treated group) and control group ($p \leq 0.05$) as shown in table (1).. Additionally no significant variations were observed in the levels of sLDL in the Asp treated groups when the comparison with control group.

Through the results of our current study, we notice that there is no significant difference in serum (TC, TG, HDL, LDL,vLDL) in (Asp treated group) comparison with control group, in agreement with results recorded by Sharma et al.,[49] and Moreover, Osfor & Elias[50] reported the same effect for 12 weeks. also his is consistent with other studies shown that administered a over dose of 500 mg aspartame and the results showed no significant change after 2 weeks’ in blood cholesterol and triglyceride. caused a decrease

in lipid peroxidation, plasma cholesterol, triglycerides, and low density lipoprotein cholesterol, and an increase in high-density lipoprotein cholesterol [51]. On the other hand disagree with,Prokić et al that referred to chronic exposure to aspartame induced changes in lipid metabolism and could be involved in the development of hypercholesterolemia [52]. Dhingra et al also mentioned that: of the 20 randomised controlled experimental studies analysed, aspartame consumption had no effects on triglycerides or cholesterol concentrations for periods ranging from (13 to 28) weeks. Compared to a caloric sweetener (sucrose or fructose), of five studies, two showed a modest significant, improvement in lipid profile (TG and/or total cholesterol) in the group that received aspartame, still with no differences compared to the placebo[36].

Also from the results of our work, sorbitol was shown significantly increase in Serum triglyceride (TG) and vLDL levels in comparison with control group ($p \leq 0.05$), this may agree with jang et al., mention of ''The long-term consumption of artificial sweeteners might induce atherosclerosis via modifying Apo A-1 and cause protein cleavage, which is associated with loss of antioxidant ability and impairment of phospholipid binding ability[53.] Apo A1 structure modification could lead to the production of dysfunctional HDL-cholesterol[54]''

The results disagree with Hamdy et al., [55] its indicated that sucrose or aspartame was significantly increased level of cholesterol and triglycerides during the experiment in comparison to untreated group. While similar results were also described by Singleton et al. [56] who mentioned that Serum triglycerides increased after consumption of the drink sweetened with glucose and fructose, but not aspartame, indicating that unlike glucose and fructose, aspartame does not enhance postprandial lipemia following lipid loading while Marko et al.,[57] study revealed that (treatment with Asp) caused an increase in the concentrations levels of cholesterol, LDL-cholesterol, as well as a decrease in the levels of serum HDL-cholesterol, As for Mohammed m et al.[58]

The mechanism of hypo-cholesterolemic and hypo-lipidemia effect may be backed to reduced total cholesterol synthesis by the saccharin repressed in vivo liver enzymatic activity of acetyl-CoA synthetase citratelyase; and mitochondrial citrate exchange leading to a reduction of available cytoplasmic acetyl-CoA, which is required for the synthesis of cholesterol and fatty acids **[59]**. high levels of lipids are associated with atherosclerosis and predispose to cardiovascular disease **[60]**. increase level of HDL-C is associated with fewer problems with cardiovascular diseases and vice versa. It is very clear that an increase in HDL-C level could potentially contribute to reversal of process of atherosclerosis, this is because high level of HDL-Cholestrol protects endothelial cells from the cytotoxic effects of oxidized LDL-C **[61].**it represents the ability of HDL-C to protect against heart disease. On the other hand oxidized atherogenic lipoprotein, namely oxidized LDL-C is taken up by immune system cells, which becomes engorged to foam cells. This foam become trapped in the wall of the blood vessels and contributes to the formation of atherosclerosis plaques that cause arterial narrowing and lead to heart diseases,therefore HDL-C has antioxidant and anti-inflammatory effects that prevent the atherogenic formation**[62,63]**

The significant body weight losses with high sweetener may be a consequence to the hypo-triglyceredemia and hypo-cholesterolemic effect as revealed by a decrease in total serum cholesterol especially with the high dose treated groups [64,65]. This results are in agreement with the results obtained by Dib et al.[66] who reported a significant reduction in body weight of rats [50%] and lipid levels after administration of a 14-day artificial sweetener. Though, the present results are in contrary with that obtained by Polyák et al.[67], als act on accelerated bile excretion of cholesterol metabolites and increased the fecal excretion of the cholesterol, triglycerides, neutral lipids, and phospholipids thus, the liver and plasma lipoprotein lipid contents including, cholesterol, triglycerides, and LDL-cholesterol were markedly reduced by . Thus its act as antihyperlipidemic, so it consider of health benefit[68]

Moreover liver acetyl-CoA carboxylase, phosphatidate phosphor-hydrolase, and (glycerol-3-phosphate acyl trans-ferase) activities were markedly reduced. Suppression of these enzymes would lead to a reduction of triglyceride synthesis. Cyclic AMP (cAMP) is formed from ATP by adenylyl cycles at the inner surface of cell membranes and acts as an intracellular second messenger.**[69],** c-AMP activates phosphorylase that triggers glycogenolysis, gluconeogenesis so induce hyperglycemia. Also adenylat cyclase, activates hormone-sensitive lipase that produces lipolysis and converting triglyceride into free fatty acid and glycerol.**[69]**

The increasing health awareness among people has led to the growth of the sweetener industry. However, despite approvals from various regulatory bodies some section of the audiences is still wary of using them. The adverse effects seen in animal studies are hard to ignore. Although ''natural'' does not mean safe, healthy or non-toxic the safety concerns over artificial substitutes has led to an increased demand for plant based alternatives.[70]

Table 1: Serum lipid profile concentrations in all studied groups

Groups	No.	TC (mg/dl) Mean ±SD	TG (mg/dl) Mean ±SD	HDL (mg/dl) Mean ±SD	LDL (mg/dl) Mean ±SD	VLDL (mg/dl) Mean ±SD
Sor	18	70.83^{a}±9.77	57^{a} ±18.54	31.83^{a}±7.73	27.6^{a}±12.79	11.4^{a}± 3.71
Asp	18	60^{a}±8.51	38.5^{b}±10.26	41.67^{a} ±10.39	10.63^{b} ±5.31	7.7^{b}± 2.05
Cont.	18	64.83^{a} ±5.58	33.5^{b}±2.63	43.5^{a} ±11.19	14.63^{b}± 6.62	6.7^{b} ±0.53
LSD	18	10.98	16.62	13.32	11.95	3.32

Note: Each value represents mean ± S.D values with non-identical superscript (a, b or c …etc.), were considered significantly differences ($P \leq 0.05$).

-No: Number of Cases.
-S.D.: Standard deviation.
-LSD: Least Significant Difference.
-Cont.: Control group.
-Sor: Rats group treated with overdose of Sorbitol.
-Asp: Rats group treated with overdose of Aspartame.

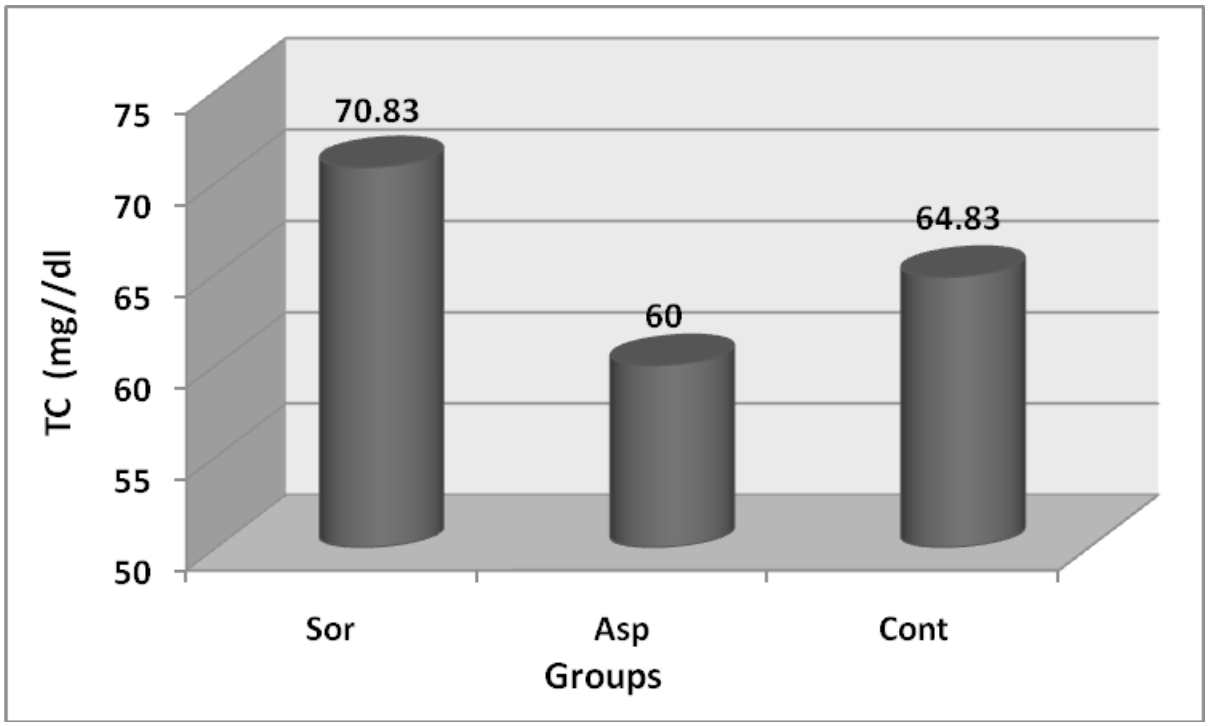

Figure (1): Serum TC levels in all studied groups

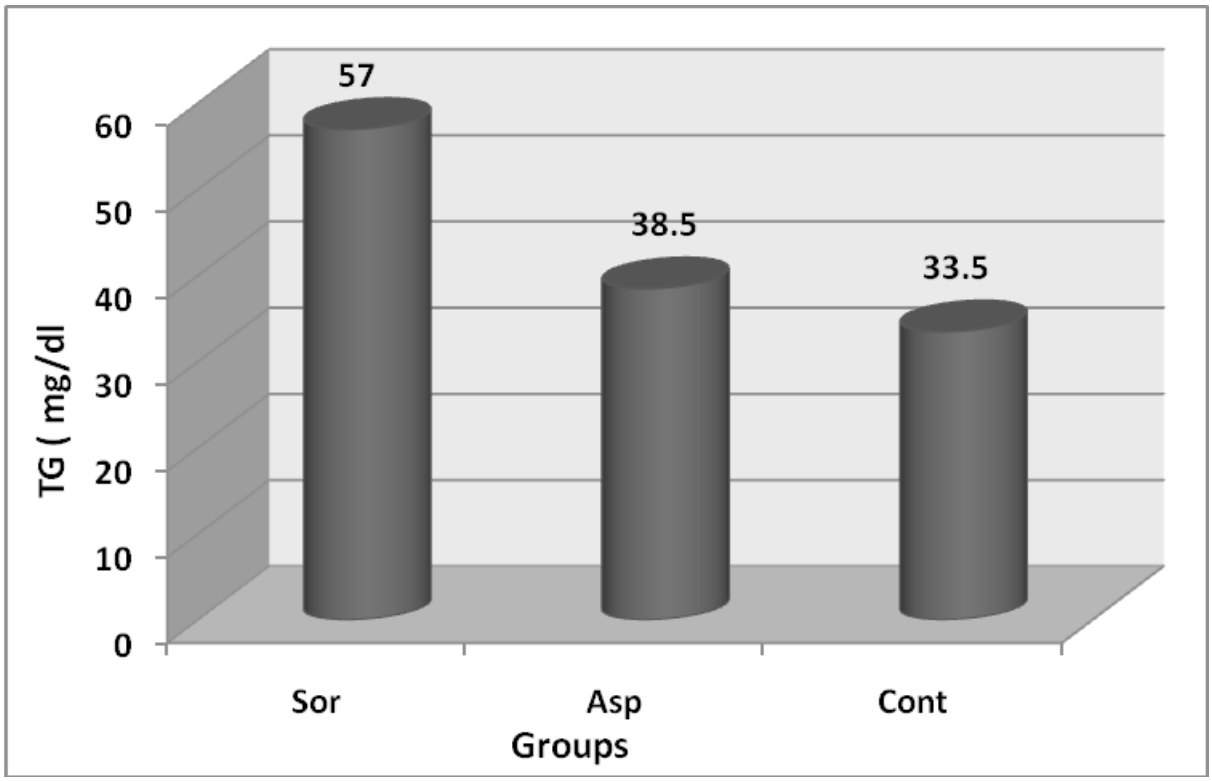

Figure (2): Serum TG levels in all studied groups

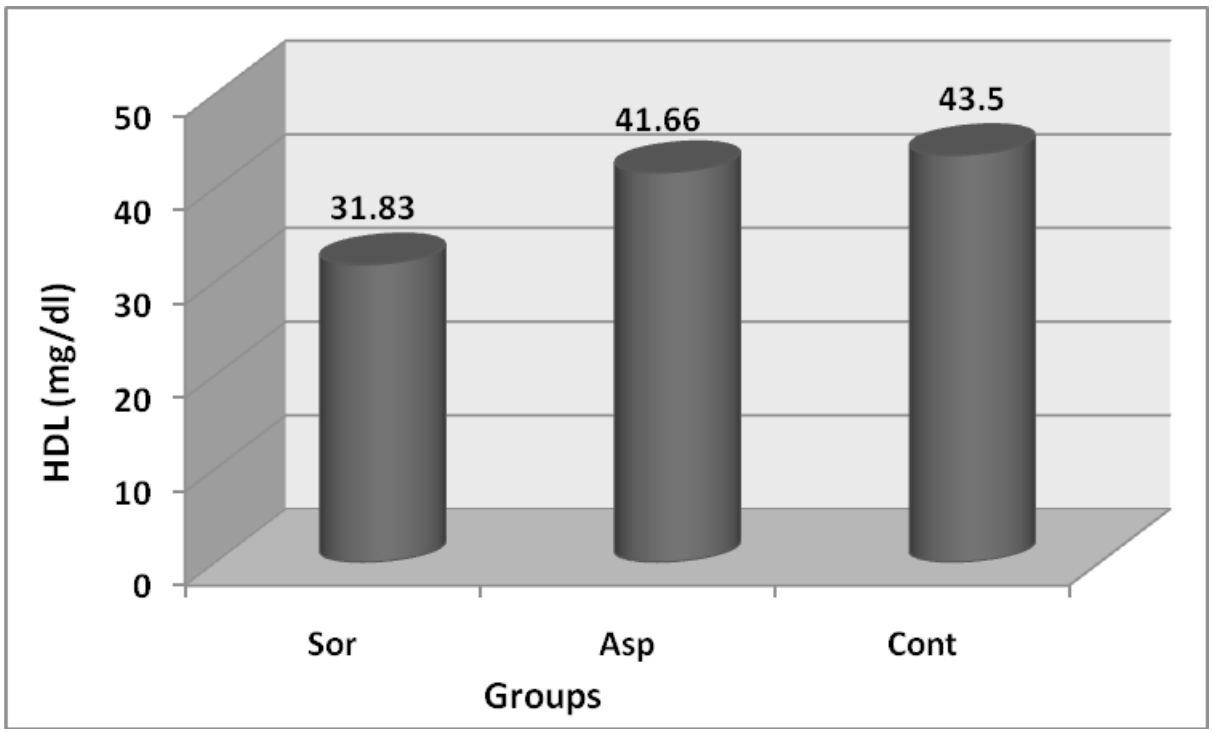

Figure (3): Serum HDL levels in all studied groups

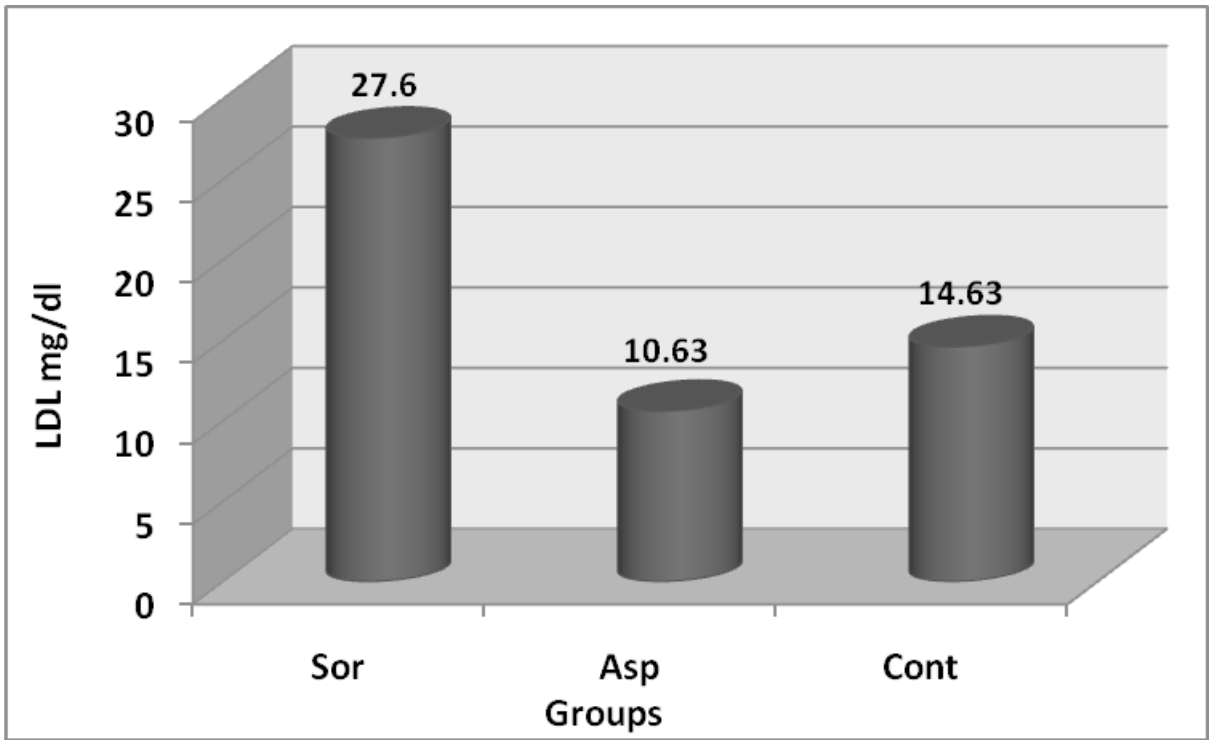

Figure (4): Serum LDL levels in all studied groups

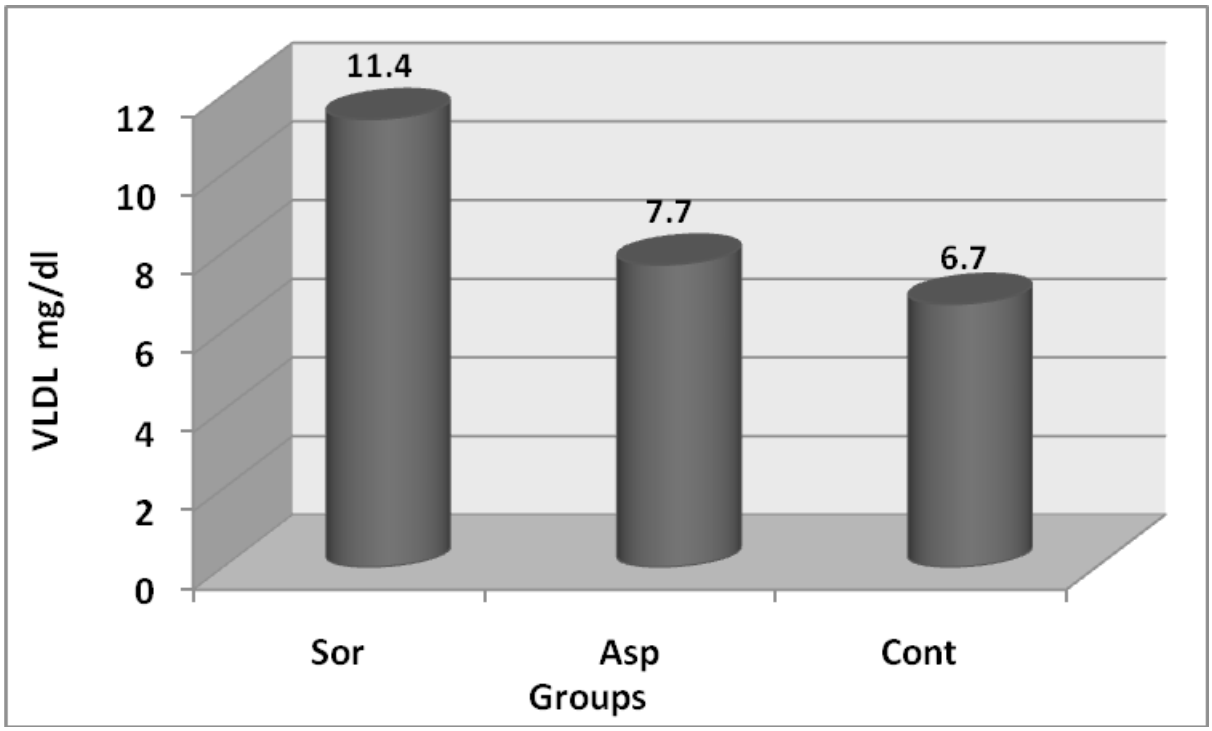

Figure (5): Serum VLDL levels in all studied groups

Conclusions

The use of overdoses than permissible of sorbitol and aspartame for a prolonged period leads to disturbance of lipid profile levels, but it is statistically imperceptible.

Ethical Clearance and financial support: Lastly the ethical approval for this study was issued by the ethical commettee of college of science of Thi-Qar university. Moreover there was a financial support from college of science in Thi-Qar university.

Conflict of Interest: Nil

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