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Research Article

EFFECT OF LONG-TERM CONSUMPTION OF ASPARTAME ON BODY WEIGHT, BLOOD GLUCOSE, LIPID PROFILE, AND KIDNEY AND LIVER FUCTION IN RATS

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A B S T R A C T

Background and objective: Aspartame (L-aspartyl L-phenylalanine-1- methyl ester) (APM) is the most widely used nonnutritive synthetic sweetener added to a wide variety of food products and drugs that are consumed by about 70% of the population. Based on the available studies, the data concerned on its effects on health and problems that may arise after its consumption remain controversial .This study was planned in order to shed a light on how chronic consumption of APM may affect important biochemical parameters as body weight, total protein, albumin, blood glucose, lipid profile, aminotransferases, and biomarkers of oxidative stress focusing in catalase in liver, brain 8-OHdG and urinary isoprostane and kidney functions (creatinine and uric acid).

Methods: Male Wistar rats were divided randomly into four groups: Group 1 as control and groups 2, 3 and 4 treated with aspartame at 20, 40 and 80 mg/kg respectively during four months. Samples of blood for measure (total protein, albumin, glucose, lipid profile, ALT, AST, LDH, creatinine, and uric acid), catalase in liver, brain 8-OHdG and urine (isoprostane) were collected every 8 weeks to proceed the evaluation.

Results: Administration of APM significantly affected all the studied parameters in all doses tested. After 4 months of treatment with APM at 40 and 80 mg/kg, respectively, there was an increased level of the following parameters: blood glucose (21 and 25) %, total cholesterol (23 and 42)%, triglycerides (24 and 55)%, LDL- cholesterol (97 and 132) %, and uric acid (53 and 48)%. The level of HDL-cholesterol was decreased around (64 and 67%. The activity of AST (36, 27 and 38) %, catalase (36, 36 and 40 % and LDH (136, 168 and 148) % were increased after 4 months of treatment at the doses of 20, 40 and 80 mg/kg, respectively. Similarly, the level of 8- OHdG (8-hydroxy-2' -deoxyguanosine), a known biomarker for oxidative stress and carcinogenesis, was increased to about (55) % after 4 months of treatment with APM at 80 mg/kg. Function of the kidney was affected by the treatment with APM during 4 months, as showed by the significant increase in creatinine (20, 104 and 31) % at 20, 40 and 80 mg/kg. Finally, level of urinary isoprostane, a prostaglandin-like compound produced by the free radical mediated peroxidation of lipoproteins, was also elevated.

***Conclusion*:** the administration of APM during 4 months showed a deleterious effect on the kidneys and liver function. Besides that, a combination of atherosclerotic effects were also observed, as well as, a general increased exposure to oxidative stress, as demonstrated through the 8-OHdG and Urinary isoprostane levels elevation. The sweetener consumption increased the liver lipoperoxidation and the risk of carcinogenesis. The kidney's function was also affected. Therefore, APM was considered unsafe to be included in the diet. Taken together, these results suggest that the long-term consumption of APM may be harmful in humans.

INTRODUCTION

Artificial sweeteners are also called sugar substitutes, alternative sweeteners, or non-sugar sweeteners. They are substances used to replace sugar in foods and beverage. Once high sugar consumption is associated with dental caries, obesity, and cardiovascular disease, which occur because of rapid increases of sugar levels leading to deleterious effects, especially in patient with diabetes. [1, 2].

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Artificial sweeteners can be divided into two large groups: nutritive sweeteners, which add some calories to the food; and non-nutritive sweeteners, which are also called high-intensity sweeteners because they are used in very small quantities, adding no energy value to the food [1].

Artificial sweeteners consumption may cause migraines or headache, skin eruptions, muscle dysfunction, depression, weight gain, liver and kidney toxicity, multiple sclerosis, blurred vision, respiratory problems, cancers, allergies, seizures and immune system dysfunction [3, 4]. Also can profoundly influence diabetes related parameters and its related complications [5].

Aspartame is a dipeptide derivative (L-aspartyl L-phenylalanine methyl ester) that is used in foods and beverages worldwide. [6], and was first discovered in 1965 by James M. Schlatter. [7, 8].

Since its approval aspartame has been used in over than 6000 different types of products including; soft drink, jams, chewing gum, canned fruits, candies, cosmetic products, vitamins, and medications. [9, 10]. Sweeteners are also widely used in the weight loss regime. [11].

Similarly as sugar and protein, aspartame produce 4 calories/g when metabolized [12, 13], and it is approximately 180 times sweeter than sucrose. It was considered to be safe for human consumption by more than ninety countries worldwide, and by over 100 regulatory agencies [7].

The acceptable daily intake (ADI) value for aspartame determined by Joint FAO/WHO Expert committee on food Additive (JECFA) and the European Commission Scientific Committee on Food has determined this value is 40 mg/kg [14], while Food and Drug Administration (FDA) has set its ADI at 50 mg/kg [15].

After its oral ingestion, aspartame is absorbed from the intestinal lumen and hydrolyzed to phenylalanine (50%) –the precursor for two neurotransmitters belonging to the catecholamine family; aspartic acid (40%), an excitatory amino acid; and methanol (10%), which is oxidized to cytotoxic formaldehyde and formic acid [16, 17, 18, 13]. After aspartame consumption, the concentration of its metabolites are increased in the blood [19].

Although the FDA approved aspartame consumption, its use has been controversial as it has been associated with several adverse effects as hyperglycemia [20, 21], neurologic and behavioral disturbances [22,11], hepatocellular lesions [23], neurotoxicity [24, 25, 26, 27], genotoxicity [28, 29], carcinogenicity [30, 31, 32, 6, 33] and potential toxicity [10, 34, 24, 25]. In addition, could be a risk factor for brain cancer, multiple sclerosis and epilepsy [6].Aspartame cause kidney dysfunction, hematological changes, and oxidative stress in rats [35]. Most of those effects were related to the generation of aspartame metabolites, particularly to methanol metabolites as formaldehyde and formate [36].

A short-term study conducted in rats demonstrated that administration of aspartame could alter blood biochemistry parameters levels [37, 38].

Several studies suggested that long term aspartame administration may be responsible for oxidative stress and hepato-renal toxicity [39, 37, 40, 23, 38, 41].Moreover, aspartame can lead to generation of oxidative stress in different brain regions [42, 11, 23, 39, 43, 44, 45], in immune organs (spleen, thymus, lymph nodes and bone marrow) in rats [46], and also may cause oxidative stress in the spinal cord [47].

The biomarker 8-OHdG or 8-oxodG, has been established as a commonly measured and sensitive marker of DNA damage. It was considered a very important biomarker not only for carcinogenesis but also for aging and degenerative diseases [48].

Isoprostanes are prostaglandin-like compounds that are produced by the free radical mediated peroxidation of lipoproteins [49]. The discovery of Isoprostanes has important implications for medicine [50]. It has been established that measurement of F2-isoprostane is the most reliable approach to assess oxidative stress status, providing an important tool to explore the role of oxidative stress in the pathogenesis of many diseases [51].

There are still many concerns raised about the side effects of aspartame consumption and if it can be used safely. Intensive searches showed that no studies have yet tested the effect of the long term intake of aspartame in different doses, and including the evaluation of different biochemical parameters in order to a better understand its effect. Therefore, the present study was planned.

MATERIALS AND METHODS

Animals

Male Wistar rats (3-4 months), weighing 250-325g were purchased from the animal house of the College of Veterinary Medicine, University of Duhok. These animals were kept in ventilated cages at controlled temperature (22 C^{o}±2) and cycles of light and dark. Food and water were given ad libitum. Rats handling and treatment was according to guidelines for laboratory animal care and use [52].

The study was approved by the Animal Ethics Committee of the College of Veterinary Medicine, University of Duhok.

Biochemical assays

MATERIALS

Aspartame was purchased from Alfa Aesar Thermo Fisher Scientific, Germany. Glucose, total cholesterol (TC), triglyceride (TG), HDL- cholesterol (HDL), uric acid, creatinine, total protein(TP), albumin, alanine transaminase(ALT), aspartate transaminase(AST) and lactate dehydrogenase(LDH) kits were purchased from Biolabo, France. Isoprostene and 8-OHdG were purchased from MyBioSource.USA.

METHODS

Enzymatic methods were applied to measure the level of glucose [53], total cholesterol [54], and triglycerides [55]. The level of HDL-cholesterol was measured according to Burtis etal (1999)[56]. LDL-cholesterol was calculated according to Friedwald formula [57], as follows:

LDL-C(mg/dl) = TC-(HDL-C+ VLDL-C)

Total protein was determined by the method of Biuret [58]. Serum albumin level was measured using bromocesol green reagent according to Doumas *et al* (1971)[59].

Serum creatinine level was assayed according to the Method of Jaffes using alkaline picrate reagent [60]. The level of uric acid was quantified as described by Fosatti etal (1971)[61]. The activity of AST and ALT was assayed according to the method of Reitman and Franke(1957)[62]. LDH activity was quantified using the Method of Youngs (1995) [63].

The activity of serum catalase was assayed according to Hadwan and Abeds (2016)[64]. Finally, Isoprostane and 8-OHdG were determined by double- sandwich Elisa method Morrow *et al*., (1990) and d Souza-Pinto *et al* (2001) (65,66].

Experimental Design

Rats were divided into four groups (10 animals/group); Group 1 as control group (food and water ad libitum), and Groups 2,3 and 4 received the administration of aspartame 20,40,80 mg/kg, respectively. Aspartame was daily prepared and dissolved in D.W. as 3ml/kg.b.w. All treatments were given orally once a day via gavage needle for 4 months.

Samples collection

Blood samples were collected at zero time and after 2 and 4 months from the orbital plexus vein [67]. The serum was separated and used for the measurement of the biochemical parameters. Liver and brain tissue was removed after 4 months and used for the assay of catalase activity and determination of 8-OHdG level. Urine samples were collected at zero time and after 2 and 4 months of treatment to the dosage of isoprostane.

Statistical analysis

All data were analyzed by-one-way analysis of variance (ANOVA).

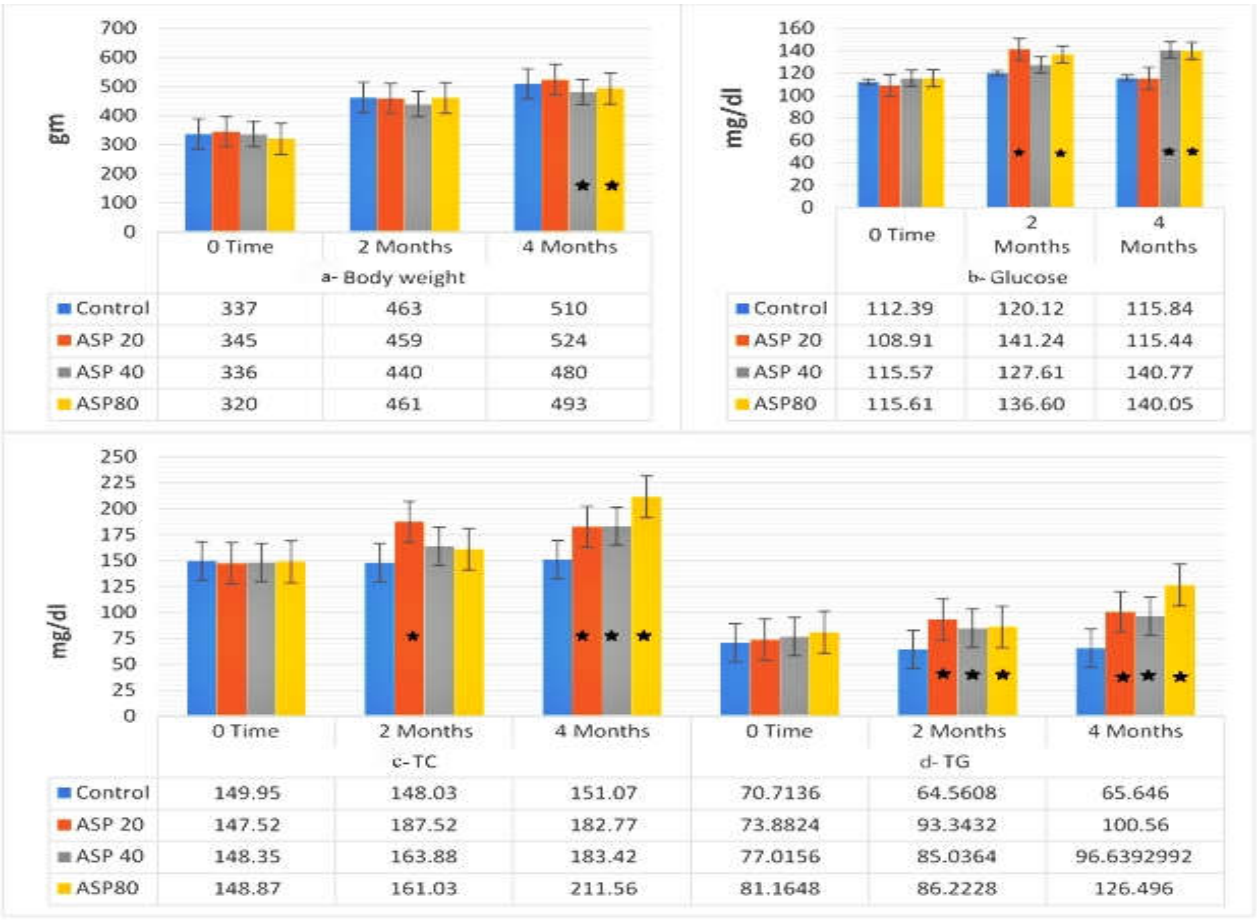
Specific group differences were determined using post-hocmultiple range test [68].

The accepted level of significant was $P<0.05$. Values were expressed as mean ± SEM of (7-10) rats per group.

RESULTS

The effects of aspartame consumption at different doses on body weight and serum glucose were presented in Figure (1:a,b).There was a significant decrease in body weight observed at doses of 40 and 80 mg/kg after four months of treatment. Aspartame consumption significantly increased blood glucose level ($P<0.05$), in a dose and time dependent fashion. Only the animals treated with 20 mg/kg returned to the normal level of glucose after 4 months.

This same effect was observed for aspartame intake on TC, TG and LDL-cholesterol; all doses significantly increase the above-mentioned lipid parameters with a highest increase in the group treated with 80 mg/kg especially after 4 months of consumption. Regarding to HDL-cholesterol, APM consumption leaded to a significant decrease in its level in those rats treated with 40 (19.4 ± 1.08) and 80 mg/kg (22.3 ± 1.71) mg of APM after 4 months. The decrease of HDL-cholesterol was inversely proportional to the period of consumption (Figure 1: c, d) (Figure 2: a,b).



	a- Body weight			b- Glucose		
	0 Time	2 Months	4 Months	0 Time	2 Months	4 Months
Control	337	463	510	112.39	120.12	115.84
ASP 20	345	459	524	108.91	141.24	115.44
ASP 40	336	440	480	115.57	127.61	140.77
ASP80	320	461	493	115.61	136.60	140.05

	c- TC			d- TG		
	0 Time	2 Months	4 Months	0 Time	2 Months	4 Months
Control	149.95	148.03	151.07	70.7136	64.5608	65.646
ASP 20	147.52	187.52	182.77	73.8824	93.3432	100.56
ASP 40	148.35	163.88	183.42	77.0156	85.0364	96.6392992
ASP80	148.87	161.03	211.56	81.1648	86.2228	126.496

Figure 1: Effect of AMP 20, 40, and 80 mg/kg.b.w. on: a- Body weight, b -Glucose, c- TC, d- TG
Comparison and analysis were done by the one-way analysis of variance (ANOVA) (n=7-10)
Data from various groups are presented as bar diagram with mean ± SEM
Significant fixed at P<0.05
Significant is marked as *

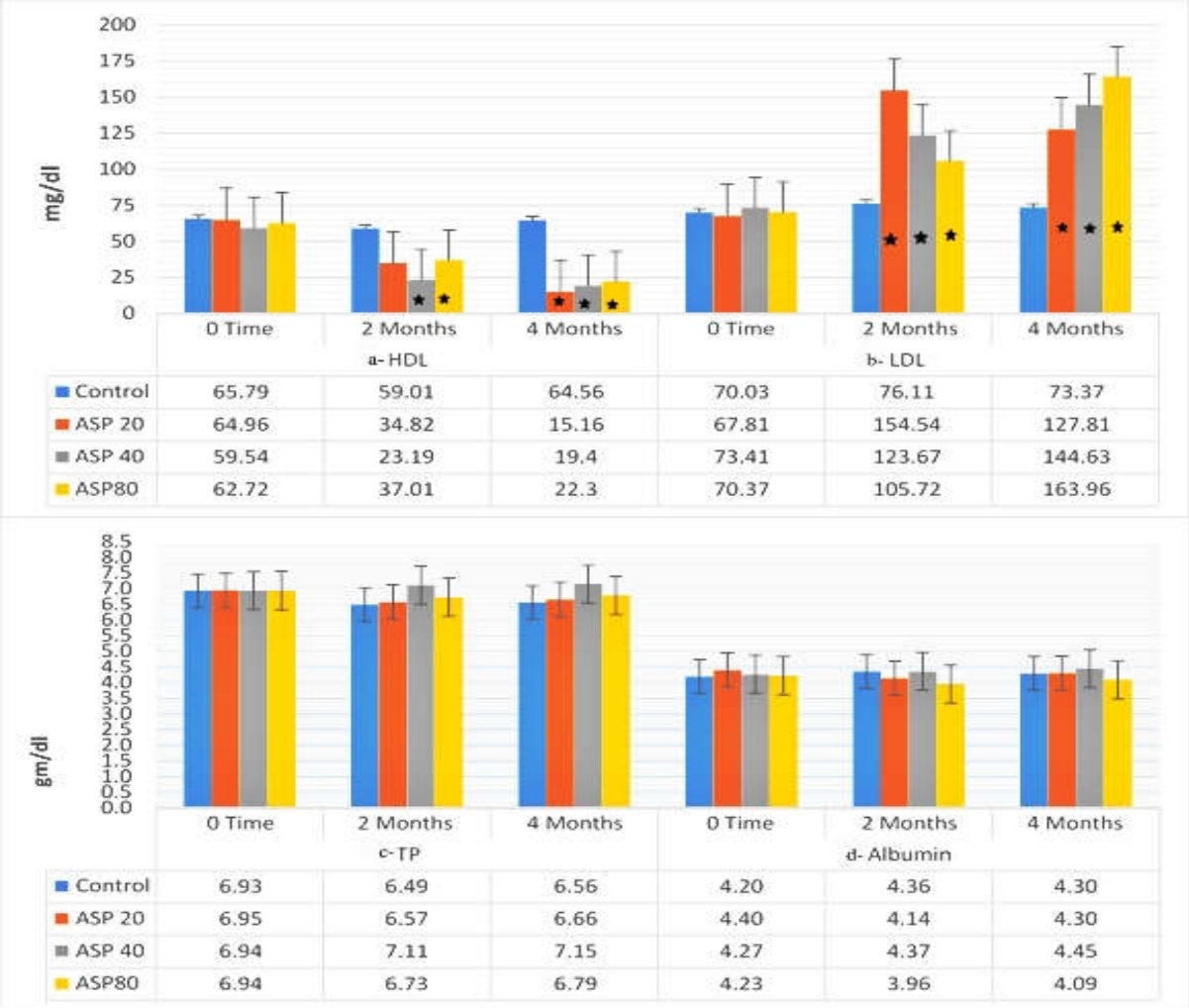


Figure 2: Effect of AMP 20, 40, and 80 mg/kg.b.w on: a- HDL, b- LDL, c- TP, and d- Albumin
Comparison and analysis were done by the one-way analysis of variance (ANOVA)(n=7-10)
Data from various groups are presented as bar diagram with mean ± SEM
Significant fixed at P<0.05
Significant is marked as *

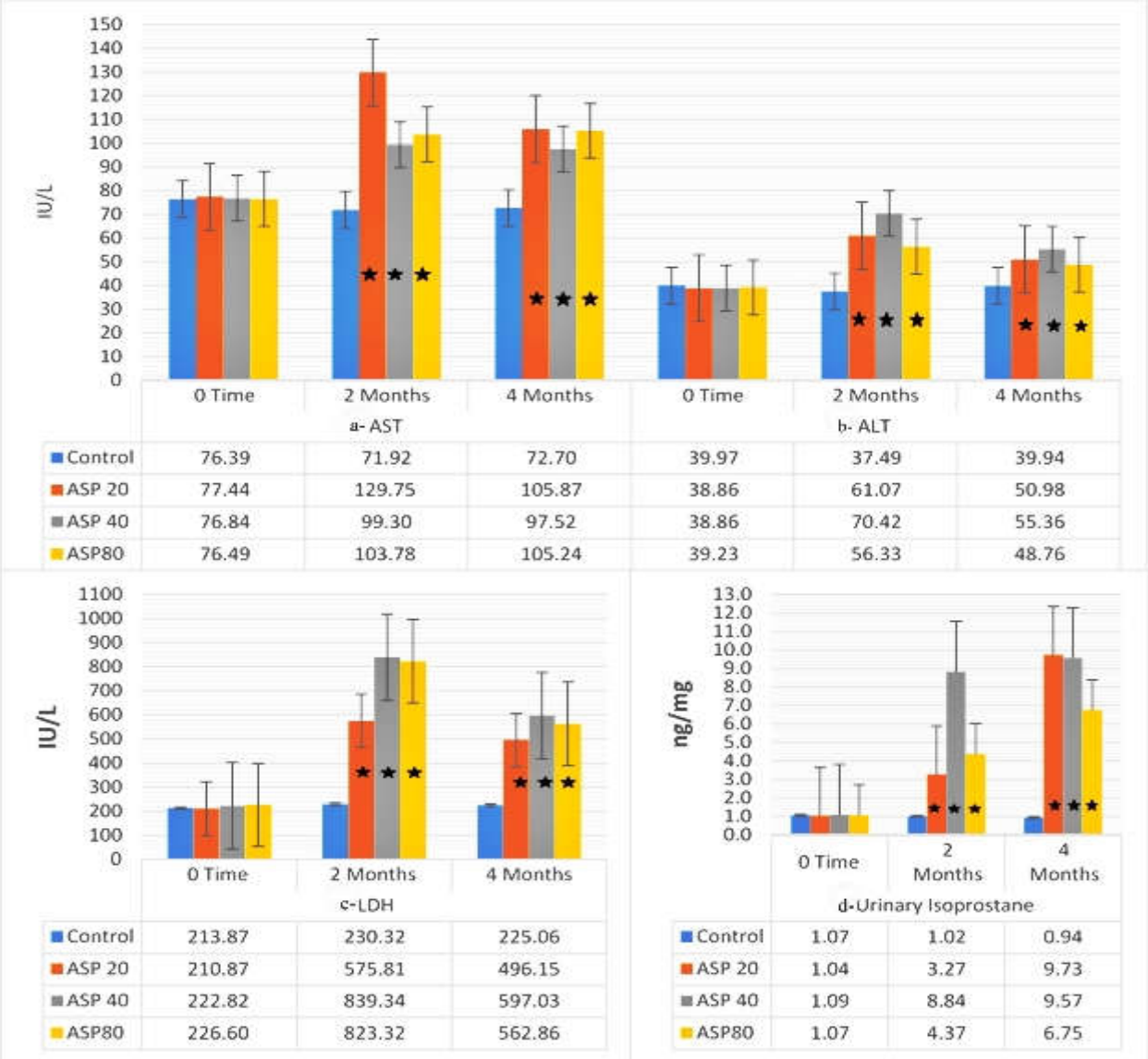
The effect of aspartame consumption at 20, 40, 80 mg/kg from zero to 2 and 4 months where shown in (Figure 2: c, d). No effect was observed on the amount of total protein and albumin levels in all doses tested, and during all over the study period.

Whereas after 2 months of APM consumption, the activity of LDH was increased in the groups treated with 20 (575.81 ± 9.32), 40 (839.34 ± 29.58), and 80 mg/kg (823.32 ± 18.78) of APM, when compared with the control group (230.32 ± 6.7). The activity of ALT was also increased in the groups treated with 20(61.07 ± 2.22), 40 (70.41 ± 4.1) and 80 mg/kg (50.13 ± 1.42) of APM, in comparison with the control group (37.49 ± 1.5). Similarly the activity of AST was increased in the animals treated with 20(129.74 ± 4.65), 40 (99.3 ± 5.31) and 80 mg/kg (103.78 ± 5.1) of APM, compared to the control group (71.92 ± 4.4). However, a decline in the activity of ALT and AST was noticed after 4 months of APM consumption in all the studied groups. The activity of LDH showed a dose dependent relationship with APM. The activity of LDH was increased in the presence of increasing doses of APM, when compared to the control group.

The treatment with aspartame caused a higher effect on the activity of AST, when compared with the activity of ALT. (Figure 3: a, b, c)

The results obtained using different doses of APM during zero, 2 and 4 months on creatinine and uric acid in serum, catalase in the liver and 8-OHdG in the brain and urinary isoprostane were demonstrated in the (Figure 3: d, Figure 4). All the treated groups showed an increased concentration of creatinine, The highest increase of creatinine was observed after 4 months of the sweetener consumption, specifically in the animals treated with 40mg/kg (1.71 ± 0.13mg/dl of creatinine). An increase in catalase activity was also noticed after 4 months of APM intake in all doses. Uric acid level was increased proportionally to the dose consumed, compared to control.

An increased concentration of 8-OHdG (95.907 ± 4035) in the brain was observed only at the dose of 80mg/kg, after 4 months of treatment. Finally, the results show that urinary isoprostane level was proportionally increased with the dose and period of consumption as can be seen in the (Figure 3 d)



	a- AST			b- ALT		
	0 Time	2 Months	4 Months	0 Time	2 Months	4 Months
Control	76.39	71.92	72.70	39.97	37.49	39.94
ASP 20	77.44	129.75	105.87	38.86	61.07	50.98
ASP 40	76.84	99.30	97.52	38.86	70.42	55.36
ASP80	76.49	103.78	105.24	39.23	56.33	48.76

	c-LDH			d-Urinary Isoprostane		
	0 Time	2 Months	4 Months	0 Time	2 Months	4 Months
Control	213.87	230.32	225.06	1.07	1.02	0.94
ASP 20	210.87	575.81	496.15	1.04	3.27	9.73
ASP 40	222.82	839.34	597.03	1.09	8.84	9.57
ASP80	226.60	823.32	562.86	1.07	4.37	6.75

Figure 3: Effect of AMP 20, 40, and 80 mg/kg.b.w on: a- AST, b-ALT, c-LDH, and d- Urinary Isoprostane
Comparison and analysis were done by the one-way analysis of variance (ANOVA) (n=7-10)
Data from various groups are presented as bar diagram with mean ± SEM
Significant fixed at P<0.05
Significant is marked as *

DISCUSSION

People who are dieting or trying to reduce the intake of calories to get rid of chronic diseases have increased with increasing rate of urbanization. The easiest method of reducing calories is to replace the high caloric food products with sweetened ones.Aspartame, among others, is the most used artificial sweetener in the world. The increase in its consumption is leading to studies of new aspects to determine if it can be safely used and to understand better its effect on human health. The present study was planned to study the effect of long-term consumption of aspartame in different doses on biochemical parameters including oxidative stress, glycaemia, lipid profile, and liver and renal functions.

The present study showed that the administration of aspartame at 40 and 80 mg reduce the body weight. This result is supported by previous studies [21, 39, 69,70].

The decrease observed in the body weight might be a consequence of the protein wasting due to unavailability of carbohydrate as an energy source [41]. Other study showed that APM consumption trigger the secretion of glucagon-like peptide (GLP)-1 by the digestive tract, resulting in weight loss through reducing appetite and calorie intake [69]. Interestingly, the treatment with 80 mg of APM for 4 months showed an opposite result in body weight, which was increased in about 6%. Is known that the sweet taste can induce an insulin response causing blood sugar to be stored in tissues. But since there is no increase in blood sugar regarding to the use of APM, hypoglycemia can be developed leading to an increased food intake. As a result, these effects can caused body weight gain as it was previously mentioned on rats [71].

Hyperglycemia was previously reported after chronic exposure to APM in zebra fish [72] and in healthy mice [20], which is compatible to the present data that showed a significant

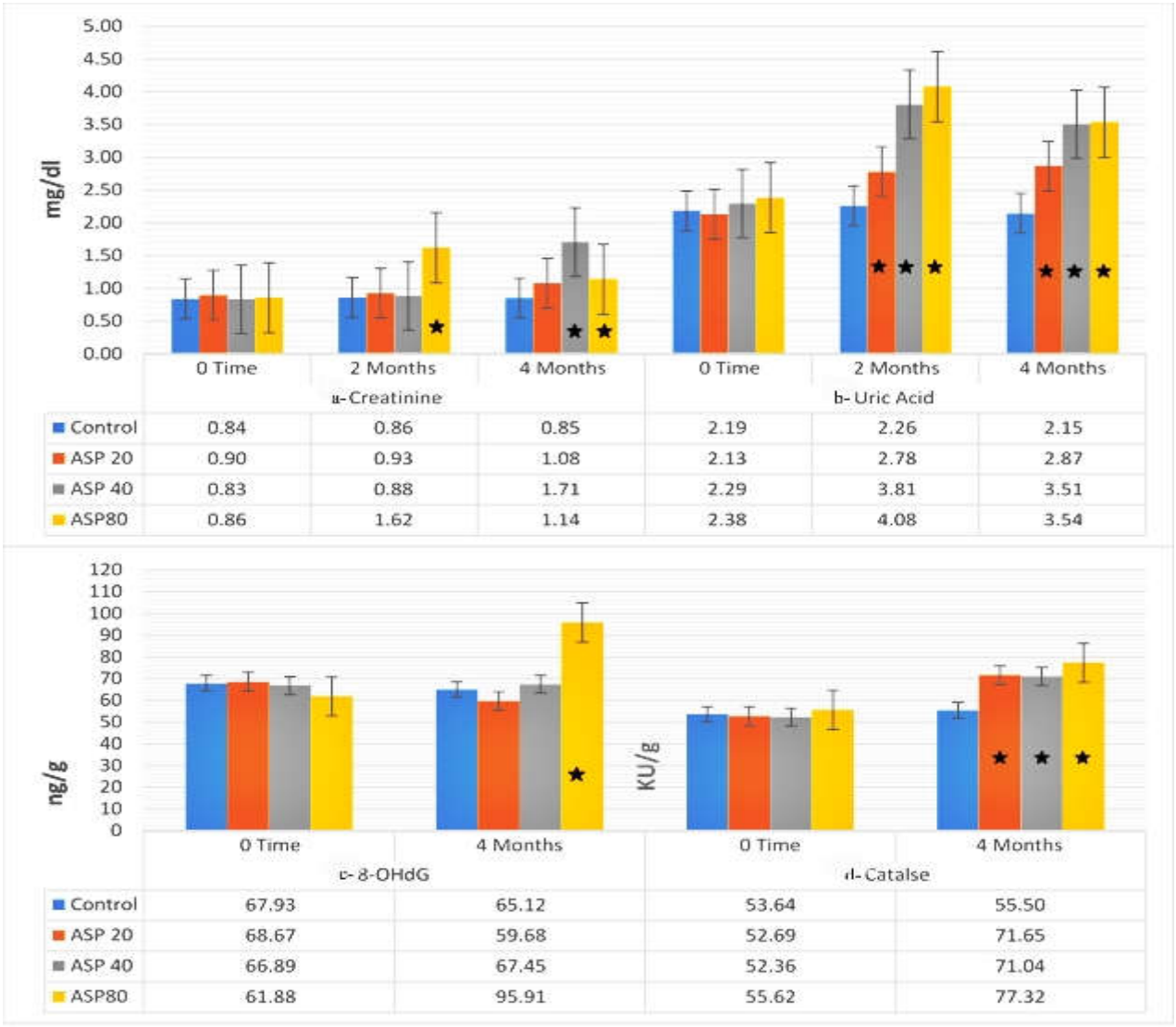


Figure 4: Effect of AMP 20, 40, and 80 mg/kg.b.w on: a- Creatinine, b- Uric acid, c- 8-OHdG and d-catalase.
Comparison and analysis were done by the one-way analysis of variance (ANOVA) (n=7-10),
Data from various groups are presented as bar diagram with mean ± SEM.
Significant fixed at P<0.05.
Significant is marked as *

increase in blood glucose in all tested groups, even those who consumed 20 mg of APM. The present study proved that the increase in blood glucose is dose and treatment period dependent. In addition to the increase in fasting blood glucose, the exposure to artificial sweeteners including aspartame, promote impairment in insulin sensitivity [73]. Moreover, it was indicated that phenylalanine, a metabolite of APM, might stimulate an increase in insulin and glucagon levels in serum of healthy individuals [74] and induce an elevation of hepatic glucose production and its level in blood of rats [21].

Regarding to the lipid profile, it was clearly indicated from the current data that TC, LDL-cholesterol and TG were significantly increased, whereas HDL-cholesterol level was significantly decreased when compared to the controls. 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 impair phospholipid-binding ability [75]. Apo A1 structure modification could lead to the production of dysfunctional HDL-cholesterol [76, 77]. Chronic exposure to APM induced changes in lipid metabolism and could be involved in the development of hypercholesterolemia [38] and atherosclerosis [75]. Hypercholesterolemic atherosclerosis was associated with increased ROS, which represent a critical initiating event in the development of atherosclerosis and cardiovascular disease [78]. Superoxide anion facilitates oxidative modification of LDL-cholesterol that plays a key role in the formation of atherosclerotic lesions [79]. The new finding in the current study is that the decrease or increase in the above-mentioned parameters was a dose and exposure period response relationship. The highest increase in TC, LDL-

cholesterol and TG occurred in the group treated with 80 mg of APM for 4 months as compared to the control group.

Oxidative stress is imbalance between oxidants and antioxidants in favor of the oxidants, potentially causing damage to cells or cellular components [80]. Particularly, destructive aspect of oxidative stress is the production of ROS, which include free radicals, H_2O_2 and peroxide that can cause extensive cellular damage [81]

Methanol, a metabolite of aspartame is primarily oxidized to formaldehyde by the formation of superoxide anion and hydrogen peroxide [82]. It was shown previously that there was a significant increase in the SOD activity after chronic APM ingestion associated with an increase in the catalase activity [11]. Catalase is the main scavenger of H2O2 at high concentration [83]. It has been found that consumption of methanol provokes changes in the activity of antioxidant enzymes with an increase in the activity of catalase [84]. The increase in the activity of this enzyme may also be due to the free radicals generation. Free radicals may also induce the expression of antioxidant enzymes, through which activating the neuronal resistance to oxidative stress [85]. This mechanism was considered accepted and came in line with the results of hepatic catalase activity in the current study [11]. Consumption of different doses of aspartame for 4 months lead to a significant increase in catalase activity in all the treated groups reaching maximum increase in its activity up to about 46% in the group treated with 80 mg as compared to the control. This result was disagreeing with other previous studies that indicate a decrease in catalase activity in the liver after APM consumption [86,87] and attributed to the increase in the methanol production.

Oxidants can cause various types of damage to DNA, such as strand breaks and oxidation of sugar and base residues [88,89]. The C-8 position of deoxyguanosine in DNA is hydroxylated to yield 8-OHdG [90]. Endogenous levels of 8-OHdG are increased upon exposure to oxidative conditions [91]. 8-OHdG has been proposed to be an indicator of oxidative damage in DNA both in vivo and in vitro [92,93,94]. The gradual significant increase of 8-OHdG in the brain with increase the treatment doses from 59.68 to 67.45 to 95.91 ng/g for 20,40 and 80 mg respectively for 4 months confirm the progression to oxidative stress resulted from APM consumption.

Under certain conditions, arachidonic acid undergo auto oxidation in vitro resulting in the formation of prostaglandin-like compounds [49]. Isoprostanes are prostaglandin like substances that are produced in vivo independently of cyclo oxygenase (COX]. Many studies have pointed out the usefulness of these compounds, particularly 8-iso-PGF2 as a marker of oxidative stress under many different clinical and experimental conditions [95,96,97]. It was mentioned previously in animal models of oxidant injury, levels of F2-isoprostane increase dramatically both free in blood and esterified to tissue phospholipids in target organs that are damaged.

Owing to this illustration, measurement of F2- isoprostane have much greater utility compared with other markers of lipid peroxidation. [96]. A significant elevation in urinary isoprostane occurred in the treated groups with all doses in the current study. Fortunately, a gradual increase in its level with the period of consumption was noticed, whereas unfortunately as it was shown in the figure (3:d) the increase in isoprostane level was not proportional to the increase in the treatment dose. That is the larger the dose resulted in a lower increase in its level.

To the best of our knowledge, these data are considered as new one since it represent the first attempt to study the effect of long term APM consumption on 8-OHdG and urinary isoprostane levels and could add supportive information to confirm the progression toward oxidative status.

Ischemic injury and oxidative stress induce a feed-forward cycle of uric acid production. Purines are liberated upon tissue injury, and with hypoxia, ATP is degraded to both adenine and xanthine. In addition, hypoxia is a potent promoter of the xanthine dehydrogenase/ xanthine oxidase holoenzyme and preferentially increases the oxidase form of the enzyme. [98] The latter uses molecular oxygen in place of NAD^+ as electron acceptor and induces the formation of superoxide anion and hydrogen peroxide in parallel with serum uric acid [99]. Although clinical and experimental evidence suggest that urate has actually antioxidant properties, it is rational that under these conditions its antioxidant activity is overcome by the pro-oxidant and proinflammatory effects of ROS accumulation [100]. Back to the results obtained in this study, an increase occurred in serum uric acid with increasing the doses after 2 months of consumption as compared to the baseline group. after that, a slightly decline in uric acid level was noticed on comparison of the results obtained after 4 months with those results obtained after 2 months. Our suggestion is that the increase could be attributed to the oxidative stress induced by APM consumption for 2 months, when the ROS overwhelmed after 4 months, uric acid level declined.

Toxic hepatitis refers to direct damage of hepatocytes by a toxin or toxic metabolite. The first laboratory abnormality to appear is an increase in prothrombin time, followed by increased activities of cytosolic enzymes. Initially, LDH is often increased to higher absolute amounts than AST, and AST tends to be higher than ALT [101]. Similarly, that is what occurred after aspartame consumption in the current study. Possibly, it was attributed to methanol, the byproduct from aspartame metabolism, which is reported to produce altered oxidant/ antioxidant balance and surface charge density causing the leakage of ALT and AST [82]. Free radical attack unsaturated fatty acids in the cellular membrane, resulting in membrane lipid peroxidation which decrease its fluidity leakage of enzymes and loss of receptor activity as well as damage membrane proteins leading to cell inactivation [23, 102, 41, 37] . the new finding in our study was that the activities of the above mentioned enzymes is independent on the dose and the period of exposure and the increase which occurred in LDH was much higher than that noticed in ALT and AST.

Finally, chronic consumption of APM cause kidney injury represented by the significant increase in creatinine level in blood. A significant increase in creatinine level noticed proportionally with the dose and the period of consumption clearly indicated in our results. These results confirmed that nephrotoxicity is the major dose- limiting side effect of aspartame as previously reported by [103,104].these changes reflect the severity of renal insufficiency associated with glomerular filtration rate because of the majority of methanol

that enters specifically the proximal tubular epithelial cells, binds to phospholipids in target cells inducing abnormalities in the function and metabolism of intracellular membranes and organelles then developed injury in the proximal tubular epithelial cells of kidney [82].

CONCLUSIONS

After discussing all of the above mentioned parameters assayed in rats, it was concluded that the intake of APM for long period even at the ADI (40 mg] has a deleterious effect on the normal functions of the kidneys and liver. As shown by the results, the kidney's glomerular function was affected. The sweetener consumption increased the liver lipoperoxidation and the risk of carcinogenesis. Besides that, a combination of atherosclerotic effects were also observed, as well as, a general increased exposure to oxidative stress, as demonstrated through the catalase, 8-OHdG, and urinary isoprostane levels elevation. Therefore, APM was considered unsafe to be included in the diet. Taken together, these results suggest that long-term APM consumption could lead to atherosclerosis, renal failure and liver cancer.

Supplementary Materials

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