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Evaluation of Oxidative Stress and Some Biochemical Criteria in Male Rabbits Following Administration of Aspartame

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Abstract | Since aspartame (L-aspartyl-L-phenylalanine-I-methyl ester, ASP) is one of 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. The present work carried out to assess whether the daily administration of ASP induced alteration in body weight and biochemical indices in male rabbits. Eighteen rabbits were used and distributed randomly into three groups, group one considered as control which was administered 1ml of distilled water orally, group two was administered 40mg/kg of aspartame orally, group three was administered 80 mg/kg of aspartame orally, the administration of aspartame continue for 45 days. Our findings revealed that the administration of ASP significantly affected all the studied parameters in both doses (low and high). After 45 days, there was body weight gain and significantly increased level of malondialdehyde (MDA) which was accompanied by a significant elevation in blood glucose, TC, TG and LDL-c. However, a significant reduction in the level of HDL-c was noticed. Additionally, a significantly increased activity of liver enzymes of ALT, AST and ALP were observed after 45 days of treatment at both doses of aspartame. Taken together, it can be concluded that the long consumption of aspartame leads to weight gain, hyperglycemia, hyperlipideamia and liver dysfunction, and highlight the use of animal models to assess important aspect of health.

Keywords | Aspartame, Malondialdehyde, FBG, Hepatic enzymes, Lipid indices

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INTRODUCTION

In the last couple of decades, growing concern about health and life quality has encouraged people to exercise, eat healthy food and decrease the consumption of food rich in sugar, salt and fat (Ardalan et al., 2017; Ab-Qayoom et al., 2023). Subsequently, the use of products such as artificial sweeteners has increased. Non-nutritive sweetener (NNS) consumption in associated with the increasing prevalence of obesity (Finamor et al., 2014; Choudhary, 2018). Non-nutritive sweetener (NNS) can replace the sugar in food and beverages, resulting in non-calorie products (Anbara et al., 2020). Aspartame (Laspartyl-L-phenylalanine methyl ester, ASP) is one of the most widely used nonnutritive sweeteners, discovered in 1965, produced commercially from the methyl ester of two amino acids, L-aspartic and L-phenyl alanine (AL- Salamy and AL-Wady, 2019; Steffensen et al., 2020). Also, it is possessing 180-200 times

the sweetness potency of sucrose and has a calorie value of 4 Kcal/g. Aspartame was approved by the Food and Drug Administration (FDA) in 1981 (Okasha, 2016). ASP is metabolized into three components, two amino acids (50% phenylalanine, 40% aspartic acid - aspartate) and 10% methanol (MeOH), aspartame produces harmful effects through its metabolites (Moubarz et al., 2018). Aspartate is an excitatory neurotransmitter and is normally found in high levels in the brain where it stimulates N-Methyl-D-aspartate (NMDA) receptors (Iman, 2011; Dang, 2019). Small amounts of ASP can significantly increase methanol concentration in the bloodstream. MeOH has a relatively low toxicity, but its metabolites are very toxic. It is a substance that damages the liver cells, where it is oxidized to formaldehyde and then to the formate (Lebda et al., 2017; Othman and Bin-Jumah, 2019). This process is accompanied by an elevation in NADH level and the formation of superoxide anion, which can be induces lipid peroxidation (Ikpeme et al., 2016). Methanol intoxication is associated with mitochondrial damage and increased microsomal proliferation, which results in the overproduction of oxygen radicals (Al-Eisa et al., 2018; Yang-Ching et al., 2022). These factors together with the excess of formaldehyde formed during acute methanol intoxication, cause a significant increase in lipid peroxidation (Finamor et al., 2017). The chronic exposure to aspartame was mentioned to cause headache, blurred vision, epileptic tits and brain tumours as well as numbness, insumnia, memory loss, nausea, slurred speech, personality changes, loss of energy, hyperactivity and hearing problems (Azeez and Alkaas, 2021). Furthermore, aspartame may adversely affect the capacity to control glucose metabolism in diabetic persons causing poor diabetic control and even may lead to precipitation of clinical diabetes in susceptible persons (AbdEl-Wahab et al., 2017). Given the side effects of aspartame and extensive use, the present work carried out to evaluate oxidative stress and biochemical profile in male rabbits not only to offer guidance on safety but also to propose use of animal models in defining health standard.

MATERIALS AND METHODS

Chemical administration

The European Food Safety Authority has confirmed acceptable daily intake (ADI) for 40 mg/kg bodyweight/day of aspartame. This ADI was approved by the Food and Drug Administration (FDA) for the European countries (EFSA Journal, 2013).

Chemical

Sweetener was selected based on the frequency of usage in the local food and drinks, aspartame ($Cl_4H_{18}N_2O_5$), molecular weight in (g/mol (294.31). It was purchased from pharmacy in Basrah city.

Animals care and experimental design

Eighteen male rabbits with an average body weight of 1200-1300 g were purchased from honor in the local market of Basrah city. They were housed in the animal house, two rabbits in each stainless steel cages, were kept at temperature (25±2 °C) good ventilation and were exposed to cycles of 12hrs light/dark. Food access and tape water was given *ad libitum* and they were acclimatized for 7 days before the onset of the experiment. Animals were randomly distributed into three groups, each group (six rabbits per gorup) was treated as following: The first group (a control group) received 1ml distilled water daily. The second experimental group received 40 mg/kg of aspartame; the sweetener dissolved in distilled water. The third experimental group received 80 mg/kg of aspartame, the sweetener dissolved in distilled water. The treatment continued for 45 days by oral route daily.

Bodyweight measurement: Rabbits were weighed two times before and after of the experiment to determine the gain in body weight. Each rabbit was placed on the electric weighing balance and recorded body weight on days (0 and 45) of the experiment.

Blood sampling: At the end of the experimental period animals were anesthetized using diethyl ether, blood samples were collected from cardiac by puncture intra-cardiac into test tubes, samples were centrifuged and the serum was collected and stored at –20°C until being used for examination of biochemicals indices.

Biochemical assays

Oxidative biomarker (Malondialdehyde: MDA) was evaluated according to (Ohkawa et al., 1979). The fasting blood glucose (FBG) was estimated according to method (Trinder, 1969), Commercial diagnostic kits were used for determination lipid profile, Total cholesterol was estimated based on the method of (Siedel et al., 1983), Triglycerides was assayed according to method of (Fossati and Prencipe, 1982), while the HDL-c and LDL-c were determined by method of (Burstein and Scholnik, 2016). ALT and AST was measured according to method described by (Reitman and and Frankel, 1957), whereas ALP was assayed depends on the method of (Kind and King, 1954).

The statistical analysis: Outcomes are expressed as means ± standard error (SE), statistical for experiment animals were evaluated using a one-way analysis of variance (ANOVA). A value at $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

According to the biochemical outcomes, the administration of aspartame resulted in increased in average of the final

body weight in both groups treated with aspartame in comparison to animals without treated with aspartame (Table 1). However, this increase was more pronounced the average of final body weight in the treated group with high dose of aspartame. Additionally, the aspartame administration caused an elevated biomarker oxidative (MDA) level at dose 40mg/kg whereas a remarkable increased in MDA level at dosage 80 mg/kg was noticed (Table 2). As outlined in the Table 3, a significant increase in liver indices (ALT, AST and ALP) were observed following aspartame treatment with both doses (low and high). Results also revealed that a significant increase in values of the blood glucose, total cholesterol, triglyceride and low density lipoprotein along with markedly lowered in high density lipoprotein value after treatment (40mg/kg) compared to to control group Table (4). The changes in blood glucose and lipid profile were more prominent with high dose (80mg/kg) of aspartame comparison to the values of control group.

Aspartame is a non-sugar sweetener used in diabetes and obesity diets. Its metabolites can cause harmful effects on the organs in associated with many complications as visual impairment, ear buzzing, loss of equilibrium, severe muscle aches episodes of high blood pressure and depression (Nali et al., 2017; Azeez and Alkass, 2021).

The present study represented that the administered non-nutritive sweetener solution of ASP to animals with both doses (low and high) led to increased body weight compared to control animals. This increase may be attributed to the aspartame -mediated increased appetite due to increased concentration of aspartame metabolite (N-Methyl-Daspartate). This metabolite up-take from circulation by the arcuate nucleus in the hypothalamus, the arcuate nucleus is main place to Neuro-peptide Y biosynthesis that stimulates de novo lipogenesis and thus promotes deposition fat and increased body weight (Beck et al., 2002; Feijo et al., 2013; Zhang et al., 2016). These results are in agree with the findings described earlier (Azeez and Alkass, 2018; Ragi et al., 2022) where they have found that using the artificial sweetener resulted in increased appetite, food consumption in addition decrease in basal metabolic rate subsequently increased body weight and obesity. This show that diets containing artificial sweeteners can lead to weight gain and obesity by interfering with the fundamental equilibrium of physiological processes mediated by taste receptors. On the other point, the phenylalanine is a precursor to neurotransmitter catecholamine that may increases food intake via hypothalamic adrenoreceptors which involved in central appetite regulation mechanism and thus stimulating appetite (Nermin et al., 2020). The data in current study are also similar to results described by a previous study (Mattes and Popkin, 2009) who have reported that ASP intake induce increase body weight due to its effect on appetite accompanied by increase in hunger ratings. While in another study (Steinert et al., 2011), it was demonstrated that intake of ASP induced satiety and decreases appetite is a result increases cholecystokinin secretion that delays the gastric emptying thereby lead to decrease body weight in the humans and animals.

We noticed a significant elevation in MDA level in rabbits received aspartame, after 45 days, which is an index of LPO. So, the observable increase in MDA level could be due to the depletion of antioxidant enzymes and elevated the free radicals production that cause to damage cell membrane and reduce membrane fluidity, these components are essential for proper functioning of the cell (Abdel-Salam et al., 2012; Finamor et al., 2017). On the other hand, hydrolysis

Table 1: Effect of aspartame on body weight in experimental groups.

Groups	Control D.W	ASP treatment dose 40mg/kg	ASP treatment at dose 80mg/kg
Initial body weight (g)	1288.2± 24.6b	1282.7± 25.7b	1288.8± 25.4b
Final body weight(g)	1290± 22.4a	1325.6± 27.4a	1400.0 ±29.6a

Outcomes are expressed as Mean ± SE. the symbol represent statistical difference at ($p < 0.05$) values compared to control animals.

Table 2: Effect of aspartame on oxidative biomarker (MDA) in experimental groups.

Groups	Control D.W	ASP treatment dose 40mg/kg	ASP treatment at dose 80mg/kg
Malondialdehyde (MDA) μ mol/L	0.76±0.033c	1.03±0.07 b	1.46±0.12a

Outcomes are expressed as Mean±SE the symbol represent statistical difference at ($p < 0.05$) values compared to control animals.

Table 3: Effect of aspartame on liver biomarkers (ALT, AST and ALP) in experimental groups.

Groups	ALT U/L	AST U/L	ALP U/L
Control D.W	62.80±0.21c	41.87±0.15c	118.29±0.33c
ASP treatment dose 40mg/kg	67.30±0.17b	43.46±0.30b	121.41±0.42b
ASP treatment at dose 80mg/kg	70.24±0.19a	50.13±0.27a	130.16±0.29a

Outcomes are expressed as Mean±SE. the symbol represent statistical difference at ($p < 0.05$) values compared to control animals.

Table 4: Effect of aspartame on Fasting blood glucose and lipid profile (TC, TG, LDL-C and HDL-C) in experimental groups.

Groups	FBG m g/dl	TC mg/dl	TG mg/dl	HLD-C mg/dl	LDL-C mg/dl
Control D.W	119.87±1.63c	148.36±1.28c	156.16 ±1.02c	40.54±1.23a	26.67±1,12c
ASP treatment at dose 40mg/kg	125.49±1.23b	150.26±1.17b	160.48±1.52b	36.37±1.44b	30.44±1,19b
ASP treatment at dose 80mg/kg	146.42±1.05a	153.48± 2.04a	163.35±1.36a	31.22±1.38c	36. 50±1.30a

Outcomes are expressed as Mean ± SE. the symbol represent statistical difference at ($p < 0.05$) values compared to control animals.

of aspartame completely occurs in the gastrointestinal tract to aspartic acid, phenylalanine and methanol, each being toxic at high levels. Furthermore, methanol is oxidized to formaldehyde and formic acid, and these metabolites are toxic. Formaldehyde is a known carcinogen that causes retinal damage, prevents DNA replication and teratogenic effects (Abhilash et al., 2013). These result are supported by previous studies (Gulec et al., 2006; Chang and Xu, 2006; Prokic et al., 2015) who have demonstrated that methanol administration cause to significantly decreased enzymatic antioxidant (SOD and CAT) and increased MDA level in the hepatic and renal tissues also in lymphoid organs of male albino rats. Additionally, Zararsiz et al. (2007) have recorded that a significant increase in MDA level in the kidney of rats after treatment with formaldehyde.

We also noticed a significant increase in the ALT, AST and ALP activities in aspartame -treated rats in both doses, but an increase in the activities of enzymes with high dose were more pronounced . This may be attributed to methanol, the byproduct from aspartame metabolism, which produce oxidant/ antioxidant imbalance, hepatocytes injury and increased permeability membrane causing the leakage of liver enzymes into blood stream (Othman and Bin-Jumah, 2019).

Regarding to the lipid profile, the current data indicated a significantly increased TC, LDL-cholesterol and TG levels and significantly decreased HDL-cholesterol level in treated animal compared to the control. This may be attributed to reactive oxygen species generation during aspartame metabolism leading to stressful effect on the liver consequently affects lipid metabolism (Mohamed et al., 2017). These finding are also consistent with results described earlier (Ediga et al., 2021) who have reported that the consumption of artificial sweeteners might induce changes in lipid metabolism that could enhance the development of hypercholesterolemia.

Based on these finding, it can be concluded that the long consumption of aspartame leads to weight gain, hyperglycemia, hyperlipideamia and liver dysfunction. Use of animal models clearly articulate that the aspartame is unsafe in the diet and must be avoided for consumption.

CONCLUSIONS AND RECOMMENDATIONS

It can concluded that the long consumption of Aspartame leads to weight gain, hyperglycemia, hyperlipideamia and liver dysfunction , Aspartame was considered unsafe to be included in the diet.

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NOVELTY STATEMENT

this study presents novel insights in to the use artificial sweeteners nonnutritive which are added to a wide variety of food products and drugs, aspartame is one of the most used synthetic sweetener despite its drawback including generation of undesirable metabolites that resulted in ROS formation and lipid per- oxidation , this work evaluates the effects of aspartame a alternative sweetener on body weight and liver function. The finding indicate that the aspartame synthetic sweetener has negative impacts on body weight and biochemical indices, this is first evaluation of artificial sweeteners (aspartame) in Basrah, Iraq highlighting adverse effects of synthetic sweetener on humans health.

AUTHOR'S CONTRIBUTION

AAA-R and MAA-R: Methodology, project administration. AAA-R: Investigation, writing original draft preparation, supervision. AAA-R and SAH: Statistical analysis and data curation. MAA-R and SAH: Resources. MAA-R: Writing review and editing. All authors have reading and agreed to the published version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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