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EFFECT OF ASPARTAME ON BIOCHEMICAL AND OXIDATIVE STRESS PARAMETERS IN RAT BLOOD

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Abstract: Aspartame (ASP) is one of the most widely used nonnutritive sweeteners. This study investigates the chronic effects of ASP on hematological and biochemical parameters, and its effects on the oxidative/antioxidative status in the red blood cells of *Wistar albino* rats. Rats were provided with ASP (40 mg/kg/daily for six weeks) in drinking water. Increased food and fluid intake was observed in the ASP-treated rats. Total body mass was significantly decreased in the ASP-treated rats. Treatment with ASP caused an increase in the concentrations of glucose, cholesterol, LDL-cholesterol, and in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), as well as a decrease in the levels of HDL-cholesterol in the serum. A significant decline in the number of white blood cells (WBC) was observed after ASP uptake. Based on the results we conclude that ASP induces oxidative stress, observed as an alteration of the glutathione redox status, which leads to increased concentrations of nitric oxide (NO) and lipid peroxides (LPO) in the red blood cells. Changes in biochemical parameters, lipid metabolism, as well as changes in the levels of oxidative stress markers and the appearance of signs of liver damage indicate that chronic use of ASP can lead to the development of hyperglycemia, hypercholesterolemia and associated diseases.

Key words: Aspartame; hematological parameters; biochemical parameters; oxidative stress; appetite

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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 (Butchko et al., 2002; Appleton and Blundell, 2007). Subsequently, the use of products such as artificial sweeteners has increased. Nonnutritive sweetener (NNS) consumption has been historically associated with the increasing prevalence of obesity (Yang, 2010).

NNS can replace the sugar in food and beverages, resulting in reduced or non-calorie products (Butchko et al., 2002). Aspartame (L-aspartyl-L-phenylalanine methyl ester, ASP) is one of the most widely used nonnutritive sweeteners, widespread in over 90 countries in about 6 000 products (Magnuson et al., 2007). It is about 200 times sweeter than sucrose. After oral administration, ASP is metabolized into three components, two amino acids (50% phenylalanine - Phe and 40% aspartic acid - aspartate, Asp) and 10% methanol (MeOH). Aspartame produces effects through its metabolites (Humphries et al., 2008).

Phenylalanine and aspartic acid are both amino acids found in natural proteins, and under normal circumstances are beneficial, if not essential, for health (Magnuson et al., 2007). Phenylalanine is a precursor in the synthesis of tyrosine (Walton, 1988), DOPA, dopamine, norepinephrine, epinephrine, phenylethylamine (Magnuson et al., 2007; Humphries et al., 2008). Aspartate is an excitatory neurotransmitter and is normally found in high levels in the brain where it stimulates N-Methyl-D-aspartate (NMDA) receptors (Chen et al., 2005).

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 increasingly recognized as a substance that damages the liver cells, where it is oxidized to formaldehyde and then to formate (Parthasarathy et al., 2006). These processes are accompanied by an elevation in NADH level and the formation of superoxide anion, which may be involved in lipid peroxidation (Castro et al., 2002). Methanol intoxication is associated with mitochondrial damage and increased microsomal proliferation, which results in the overproduction of oxygen radicals (Humphries et al., 2008). These factors, together with the excess of formaldehyde formed during acute methanol intoxication, cause a significant increase in lipid peroxidation (Parthasarathy et al., 2006; Humphries et al., 2008).

After ASP consumption, the concentration of its metabolites increases in the blood (Humphries et al., 2008). After tissue damage, some of the enzymes find their way into the serum leaking through membranes with altered permeability. Activity measurements of serum enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γGT)) are a valuable tool in clinical diagnosis. The measurement of these enzymes' activities in tissues and body fluids can be used to estimate the degree of toxicity of a chemical compound on an organ/tissues (Vijan, 2010; Abhilash et al., 2011).

Clinical studies have shown that the administration of ASP could be responsible for neurological and behavioral disturbances in sensitive individuals (Walton, 1988; Iyyaswamy and Rathinasamy, 2012). In addition, the chronic

use of ASP might contribute to hypersensitivity reactions and atherosclerosis (Walton, 1988; Butchko et al., 2002; Jang et al., 2011). The effects of long-term consumption of ASP products have not yet been discussed.

The aim of this study was to investigate the chronic effects of ASP (40 mg/kg b.w.) on hematological parameters, glucose and lipid profiles, activities of hepatic marker enzymes, and its effects on oxidative stress parameters and glutathione redox status in red blood cells of male Wistar rats.

Table 1. Effect of aspartame (ASP) on body weight, calories, food and water intake.

Parameters	Experimental groups	
	Control	ASP
Initial body weight (g)	204 ± 9.2	208 ± 16.8
Final body weight (g)	486 ± 15.7	476 ± 26.4
Gain of body weight (g)	282 ± 6.5*	268 ± 8.4
Food intake (g/rat/day)	26.2 ± 1.2	29.2 ± 1.3
Caloric intake (kcal/rat/day)	79.1 ± 3.6	88.2 ± 3.8*
Water intake (mL/rat/day)	46.0 ± 1.7	47.8 ± 1.8

Values are expressed as means ± S.E.M. (n = 5 animals). *p<0.05 when compared with the control group.

Table 2. Effect of aspartame (ASP) on haematological parameters.

Parameters	Experimental groups	
	Control	ASP
RBC (x 10^{12}/L)	5.25 ± 0.31	5.47 ± 0.12
Hb (g/L)	116.5 ± 4.15	98.3 ± 2.31
Hct (%)	38.57 ± 0.76	35.74 ± 0.27
WBC (x 10^{9}/L)	4.58 ± 0.26	3.84 ± 0.17*
PLT (x 10^{9}/L)	574.8 ± 31.5	667.2 ± 29.7

RBC: red blood cell; Hb: haemoglobin; Hct: haematocrit values; WBC: white blood cell; PLT: platelets. Values are expressed as means ± SEM, (n = 5 animals). *p<0.05 when compared with the control group.

MATERIALS AND METHODS

Chemicals

Chemicals for this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). All reagents and chemicals were of analytical grade or higher purity.

Animals and treatment

Male *Wistar albino* rats (2 months old, weighing 190-220 g) were used. The animals were kept under standard laboratory conditions (12 h light/dark intervals; 21 ± 2°C). All rats were housed in individual cages (44 x 34 x 16 cm). Standard food and water were available *ad libitum* for all groups. The University Committee of the Ethics of Animal Experimentation approved all animal experiments. The European Food Safety Authority (EFSA) established 40 mg/kg as an Acceptable Daily Intake (ADI) for ASP. The animals were divided into two groups (n = 5 per group) and treated as follows: Group 1 (control group), included rats that were provided with normal drinking water; Group 2 (experimental group) included rats that were provided with ASP (40 mg/kg b.w., *p.o.*) via drinking water for 6 weeks.

Measurements of food and fluid intake and mass gain

The control of food and fluid intake was conducted daily by subtraction of the quantity remaining (g, ml) from the quantity supplied (g, ml). The daily consumption of both groups was registered and summarized weekly. Cages were carefully monitored for any evidence of food spillage and crumbs were considered for the control of food intake. The fluid bottles were also

checked for any sign of leaking or clogging. The rats were weighed weekly at the same time in the morning. An electronic precision balance (BJ 610C, Precisa) was used for all these measurements and data collectors were blinded to group assignments.

Blood sampling

Animals were anesthetized with ether and killed by decapitation. Blood samples were collected between 8 and 10 a.m. to avoid circadian rhythm-induced changes. The blood samples were collected in tubes using K-EDTA as anticoagulant for hematological analysis, and in tubes without anticoagulants for the other analysis. Hematological and biochemical parameters were performed on the day of sacrifice.

Hematological methods

EDTA-added whole blood samples were used for hematological examination. Hematological parameters such as the number of red blood cells (RBC), white blood cells (WBC), platelets (PLT), hemoglobin (Hb) concentration and hematocrit (Hct) values, were determined in whole blood by standard methods on an automated hematology analyzer (Horiba Medical ABX Micros 60, Japan).

Biochemical methods

Blood samples in non-anticoagulant tubes were centrifuged at 5 000 rpm for 15 min and the serum was used. Measurement of biochemical parameters (serum total protein (TP), albumin (Alb), glucose, total cholesterol (TC), high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, and activities of AST, ALT and LDH) were performed on an autoanalyzer (C 8000 Architect, Germany) using diagnostic colorimetric kits (Abbott laboratories).

Oxidative stress parameters

To measure oxidative stress parameters, blood samples were centrifuged and the plasma was removed. Erythrocytes were washed three times with an equal volume of cold saline (0.9%, v/v), and 1 ml of washed erythrocytes was lysed on ice in 3 ml of dH_2O (1:3, v/v) for 30 min. Nitric oxide (NO) levels were measured as total nitrite and nitrate levels with the use of Griess reagent according to the method of Green et al. (1982). Concentrations were expressed as μmol/L erythrocytes.

The lipid peroxide (LPO) level was determined based on the reaction of products of lipid peroxidation (MDA – malondialdehydes) with TBA (thiobarbituric acid reactive substances – TBARS analysis) using method of Ohkawa et al. (1979). These results were expressed in μmol MDA/L erythrocytes using a molar extinction coefficient for MDA of $1.56 \times 10^5\ M^{-1} \cdot cm^{-1}$.

Determination of reduced and oxidized glutathione

Quantification of reduced glutathione (GSH) was accomplished using the method of Beutler (1975a) based on the oxidation of GSH with DTNB (5.5-dithiobis-2-nitrobenzoic acid). GSH concentrations were expressed as mmol/L erythrocytes. The level of oxidized glutathione (GSSG) was determined after enzymatic reaction with glutathione reductase and inhibition of GSH oxidation by NEM (N-ethylmaleimide) (Beutler, 1975b). The concentrations were expressed as μmol/L erythrocytes. The glutathione redox index (GSH RI) was calculated and expressed in arbitrary units.

Statistical analysis

All data were statistically evaluated with SPSS/13 software. The results were expressed as mean ± S.E.M. and analyzed by factorial analysis of variance (ANOVA) using a *post hoc* Bonferroni/Dunnett's multiple analysis, or by nonparametric Kruskal-Wallis test, where $p<0.05$ was considered statistically significant.

RESULTS

Effect of ASP on body weight, weight gain, calories, food and water intake

Table 1 summarizes the weight gain data for the control rats and ASP-treated rats, along with their daily ingestion of water, food and calories. Average daily food and water consumption as well as caloric intake during the experiment show increased intake in rats treated with ASP. The increase was statistically significant only in caloric intake. Fig. 1 presents the average weekly food intake during the experiment. The ASP-treated group increased food intake during the third, fourth, fifth and sixth weeks in comparison to the control group. Intake was statistically increased during the third and fourth week ($p<0.05$). The results of weekly average water intake show a slightly increased intake in ASP-treated group during the first, third and fourth weeks compared to control group (Fig. 2). Weekly changes in body weight and weight gain are presented in Figs. 3 and 4. Increase of total body weight (Fig. 3) was observed in the group treated with ASP during the first and fourth week, while the value of weight gain (Fig. 4) was detected to be significantly higher ($p<0.05$) in the ASP-treated group only in the fourth week compared to the control rats. During the first, second and sixth weeks of the experiment, a significantly lower weight gain was reported ($p<0.05$) in ASP-treated group (Fig. 4). Total body weight gain was significantly higher ($p<0.05$) in the control group compared to the ASP-treated group (Table 1).

Effect of ASP on hematological parameters

The results of hematological parameters of control and rats treated with ASP are presented in Table 2. As can be observed, there were no significant changes in the number of RBC and PLT, Hb concentration and Hct values between the treated and control groups. However, the results indicated a significant decline ($p<0.05$) in white blood cell number after ASP treatment.

Effect of ASP on biochemical parameters

The effects of ASP on the biochemical parameters in serum are shown in Table 3. The presented results show a significant increase ($p<0.05$) in serum glucose, TC and LDL-cholesterol, while the level of HDL-cholesterol was decreased in the ASP-treated group compared to the control. No significant changes in serum total proteins and albumins were found in the rats treated with ASP compared to the control group.

As expected, ASP administration significantly increased ($p<0.05$) the activities of ALT, AST and LDH in the serum compared to the control group (Table 3).

Effect of ASP on oxidative stress parameters and glutathione redox status

The effects of ASP on oxidative stress parameters in the erythrocytes of the rats are shown in Table 4. The obtained results indicated that the concentrations of NO and LPO in erythrocytes significantly increased ($p<0.05$) in the group treated with ASP compared to the control group.

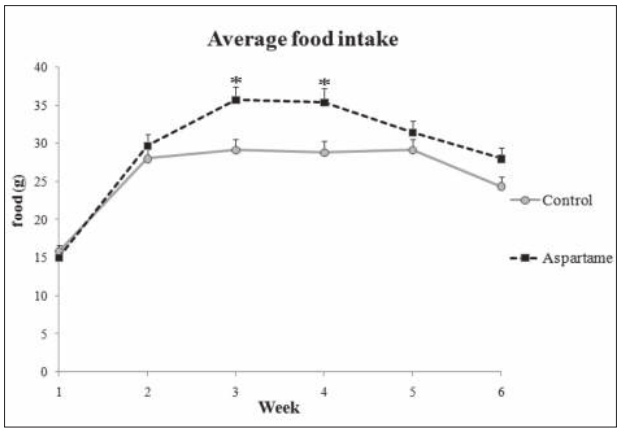

Fig. 1. Weekly change in food intake in control and rats treated with aspartame (ASP, 40 mg/kg b.w.). Values are expressed as means ± S.E.M. (n = 5 animals). *p<0.05 when compared with the control group.

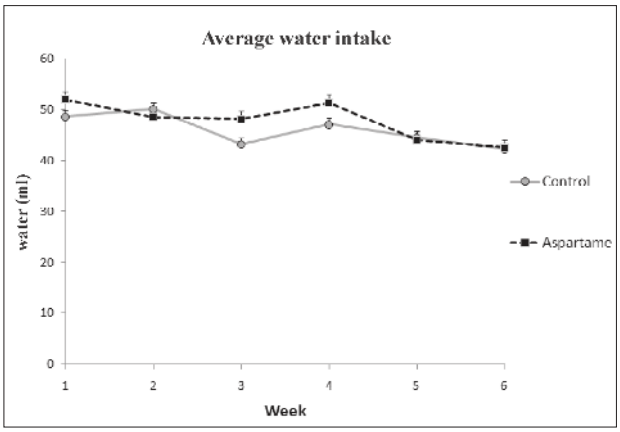

Fig. 2. Weekly change in water intake of control and rats treated with aspartame (ASP, 40 mg/kg b.w.). Values are expressed as means ± S.E.M. (n = 5 animals). *p<0.05 when compared with the control group.

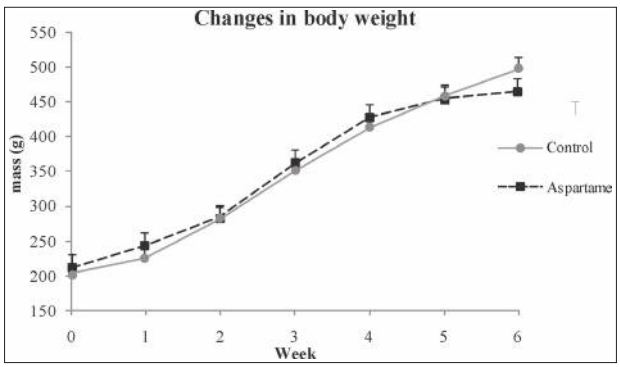

Fig. 3. Weekly change in body weight of control and rats treated with aspartame during experiment (ASP, 40 mg/kg b.w.). Values are expressed as means ± S.E.M. (n = 5 animals). *p<0.05 when compared with the control group.

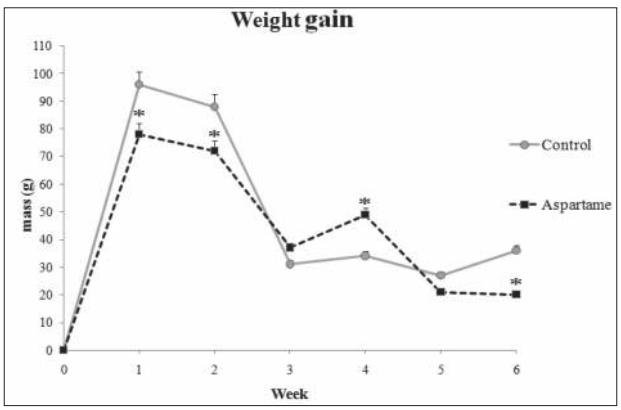

Figure 4. Weekly weight gain in control and rats treated with aspartame (ASP, 40 mg/kg b.w.). Values are expressed as means ± S.E.M. (n = 5 animals). *p<0.05 when compared with the control group.

The data presented in Table 4 show significant changes in the glutathione redox status (GSH, GSSG, GSH RI) after the ASP treatment. The concentration of GSH and glutathione redox index significantly decreased (p<0.05), while the concentration of GSSG significantly increased (p<0.05) in the group treated with ASP in comparison to the control.

DISCUSSION

ASP use has been approved by the FDA and the European Commission Scientific Committee on Food. The acceptable daily intake level of ASP established by the FDA and European Food Safety Authority is 50 and 40 mg/kg/day. People on diet and patients with diabetes,

including children, frequently use these products. In our study, we report that ASP increased appetite in rats. Appetite is a highly regulated phenomenon with hunger and satiety as crucial factors in controlling food intake. Both food intake and energy expenditure disturbances lead to obesity, a pandemic syndrome frequently associated with the most prevalent and morbid pathologies in developed countries, including heart disease, atherosclerosis, diabetes and cancer (Das, 2010). Sweetness in the absence of energy has been hypothesized to have effects on appetite because of a natural sweetness-energy association. Sweetness is naturally found in association with energy. Because of this association, it has been suggested that sweetness could cue the arrival of the associated energy and its physiological and psychological effects on appetite (Appleton and Blundell, 2007). The absence of the associated energy, as occurs in an artificially sweetened beverage, has been suggested to result in a need for energy, leading to the stimulation of appetite (Swithers and Davidson, 2008). ASP increases appetite through its metabolites by different mechanisms. An increased phenylalanine concentration releases cholecystokinin (Gibbs et al., 1976), which is an endogenous anorectic agent (Kissileff et al., 1981). As a precursor of catecholamine neurotransmitters (Wurtman et al., 1981), phenylalanine may facilitate intake via the hypothalamic adrenoreceptors implicated in the central appetite control mechanisms, stimulating appetite (Leibowitz, 1980). The ASP metabolite aspartate is taken over from circulation by the arcuate (ARC) nucleus in the brain. The ARC nucleus is the main place for the synthesis of neuropeptide Y (NPY), which stimulates carbohydrate intake (Beck et al., 2001). Increased concentrations of glutamate, or its excitatory amino acid agonists, kainic acid, AMPA and NMDA, rapidly elicit an intense food intake response in rats (Stanley et al., 1993). Aspartate is a N-Methyl-D-aspartate (NMDA) receptors agonist (Chen et al., 2005) and its increased concentration can influence food intake.

Beck et al. (2001) reported that chronic ASP use significantly decreases the concentrations of leptin in plasma. Leptin acts on the brain by inhibiting food intake, but lower concentrations of leptin could stimulate appetite (Caro et al., 1996). Increased fluid intake can be associated with the intensive sweet taste of ASP and its hedonic impact. ASP is about 200 times sweeter than sucrose (Humphries et al., 2008). Feijo et al. (2013) reported increased body weight and fluid intake in a group treated with ASP. There are opposing opinions about the effect of ASP on body weight changes. Some studies reported increased body weight caused by ASP (Feijo et al. 2013, Choudhary and Devi 2014), while others showed that ASP is efficient in body weight loss (Blackburn et al. 1997; Blackburn, 1999). We observed significantly increased appetite and lower weight gain in ASP-treated rats. The observed discrepancy suggests that ASP can affect energy (lipids and carbohydrates) metabolism, increasing energy expenditure. Other results reported in this study such as increased fluid intake, biochemical parameters in serum and oxidative stress also indicate that chronic ASP treatment can possibly lead to the development of diabetes type 2 in rats. The classic symptoms of diabetes are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), and weight loss (Vijan**,** 2010).

Treatment with ASP caused an increase in serum glucose, total cholesterol and LDL-cholesterol, while the level of HDL-cholesterol was decreased as compared to the control. Cholesterol is a major component of cell membranes.

It is essential for tissue growth and the production of steroid hormones. The liver is the major organ responsible for the regulation of total-body cholesterol metabolism (Evans et al., 1999). Similar results to those in our study were reported by Kim et al. (2011). Chronic exposure to ASP induced changes in biochemical parameters and lipid metabolism, and could be involved in the development of hyperglycemia and hypercholesterolemia. Other authors have shown that the long-term consumption of artificial sweeteners, such as ASP, might contribute to hypersensitivity reactions (Walton, 1988) and atherosclerosis development (Jang et al., 2011). Hypercholesterolemic atherosclerosis was associated with increases in ROS, which represents a critical initiating event in the development of atherosclerosis and cardiovascular diseases. Oxidative stress and oxidative damage of tissue could be the initial markers of some chronic diseases, like diabetes. During diabetes, persistent hyperglycemia causes the increased production of free radicals in all tissues from glucose auto-oxidation and protein glycosylation (Chaitanya et al., 2010).

The results presented in this study suggest that the number of WBC decreased in ASP-treated rats. The reduced WBC number is due to a redistribution of cells into damaged organs such as the liver, rather than loss of cells (Dhabhar and McEwen, 1997). Based on literature data, hyperglycemia, hypercholesterolemia and hypertriglyceridemia activate neutrophils, increasing their adhesion to the endothelium and promoting oxidative bursts (Alipour et al., 2008). These alterations may be due to the production of high levels of free radicals. Free radicals are extremely reactive and have the capacity to interact with cellular macromolecules, including lipid membranes, proteins, carbohydrates and nucleic acids, thus interfering with vital cellular functions (Halliwell and Gutteridge, 2007).

We observed a significant increase in ALT, AST and LDH activities in ASP-treated rats. The increased levels of serum enzymes indicate an enhanced permeability, damage or necrosis of hepatocytes. The disturbance in the transport function of the hepatocytes because of hepatic injury causes the leakage of enzymes from cells due to increased lipid peroxidation. It is likely that ASP induces biochemical changes in the liver. These alterations in enzyme levels may depend on exposure time and dose. Similar results were reported by Abhilash et al. (2011), Abdel-Salam et al. (2012) and Choudhary and Devi (2014).

Literature data shows that oxidative stress contributes to endothelial dysfunction by modulating NO bioavailability in the microcirculation (Pandey and Rizvi, 2010). Based on our results, ASP treatment increased the concentrations of NO and LPO in erythrocytes. The increased levels of LPO and NO are taken as direct evidence of oxidative stress (Humphries et al., 2008). This alteration after ASP administration may be attributed to its metabolite methanol. Methanol is primarily metabolized by oxidation to formaldehyde and then to formate. These processes are accompanied by the formation of superoxide anion and hydrogen peroxide (Parthasarathy et al., 2006; Humphries et al., 2008). Lipid peroxidation in cellular membranes damages polyunsaturated fatty acids tending to reduce membrane fluidity, which could lead to the release of methanol (formed during aspartame metabolism) and formaldehyde (formed during methanol metabolism). This is well supported by the report of Parthasarathy et al. (2006), who observed an increased LPO level after methanol administration in the lymphoid organs. Similarly, Mourad (2011) recorded a significant increase in LPO level in the liver and kidney of rats after chronic ASP treatment.

Table 3. Effect of aspartame (ASP) on biochemical parameters in the serum.

Parameters	Experimental groups	
	Control	ASP
TP (g/L)	64.2 ± 1.81	68.6 ± 1.41
Alb (g/L)	12.4 ± 0.7	13.6 ± 0.6
Glucose (mg/dL)	96.5 ± 14.2	138.7 ± 12.4*
TC (mmol/L)	1.24 ± 0.06	1.86 ± 0.07*
HDLC (mmol/L)	0.76 ± 0.02	0.58 ± 0.03*
LDLC (mmol/L)	0.08 ± 0.01	0.23 ± 0.03*
ALT (IU/L)	52.4 ± 2.81	74.8 ± 5.16*
AST (IU/L)	141.6 ± 4.38	186.7 ± 6.24*
LDH (IU/L)	465.8 ± 23.6	798.3 ± 54.8*

TP: total protein; Alb: albumin; TC: total cholesterol; HDLC: high density lipoprotein cholesterol; LDLC: low density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehidrogenase. Values are expressed as means ± SEM, (n = 5 animals). *p< 0.05 when compared with the control group.

Table 4. Effect of aspartame (ASP) on blood NO, LPO, GSH, GSSG and GSH RI.

Parameters	Experimental groups	
	Control	Aspartame
NO (μmol/L)	29.34 ± 1.97	53.48 ± 2.49*
LPO (μmol/L)	3.28 ± 0.20	4.59 ± 0.23*
GSH (mmol/L)	5.58 ± 0.54	3.17 ± 0.18*
GSSG (μmol/L)	1.17 ± 0.10	2.24 ± 0.12*
GSH RI (AU)	3.91 ± 0.16	2.12 ± 0.24*

NO: nitric oxide; LPO: lipid peroxidation; GSH: reduced glutathione; GSSG: oxidized glutathione; GSH RI: glutathione redox index (AU: Arbitrary Unit). Values are expressed as means ± SEM, (n = 5 animals). *p<0.05 when compared with the control group.

Reduced glutathione is a primary defense against oxidative stress. The antioxidative role of GSH is based on its ability to scavenge free radicals, to reduce peroxides and to participate as a co-substrate in the activity of GSH-dependent enzymes. The thiol/disulfide (GSH/GSSG) ratio has an important effect on the redox status of the protein thiols with modulation of protein conformation and enzyme activity. Moreover, oxygen-derived free radicals may overwhelm the radical scavenging potential of cells, leading to lipid peroxidation and the destruction of cell membranes, which in turn may result in cellular depletion of GSH, with subsequent increases of GSSG. An increased GSSG/GSH ratio is considered indicative of oxidative stress (Griffith, 1999; Shelly, 2009). The decrease in GSH activity observed in the present study could be caused by methanol, because methanol metabolism depends upon GSH (Parthasarathy et al., 2006). The depletion of GSH, an increase in GSSG and the lowering of the GSH/GSSG ratio increase cell vulnerability to oxidative stress (Shelly, 2009). These changes seem to be due to the generation of free radicals by the methanol metabolite of ASP. GSH protects the cellular system against the toxic effects of lipid peroxidation (Griffith, 1999). Usually, GSH non-enzymatically reacts with superoxide, nitric oxide, hydroxyl radical and peroxynitrite radicals (Atasayar et al., 2009).

Our results provide evidence that ASP intake induces oxidative stress in rat erythrocytes by altering the glutathione redox status. Together with the changes in biochemical and lipid parameters and indicators of liver damage, our findings suggest that chronic use of ASP can lead to the development hyperglycemia, hypercholesterolemia and associated diseases. In spite of the controversial effects of ASP, it is used worldwide by diabetics and people on different diets.

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REFERENCES

Abdel-Salam, O.M.E., Salem, N.A. and *J.S. Hussein* (2012). Effect of aspartame on oxidative stress and monoamine neurotransmitter levels in lipopolysaccharide-treated mice. *Neurotoxicol. Res.* **21**, 245-255.

Abhilash, M., Sauganth, P.M.V., Varghese, M.V. and *R.H. Nair* (2011). Effect of long term intake of aspartame on antioxidant defense status in liver. *Food Chem. Toxicol.* **49**, 1203-1207.

Alipour, A., VAN Ostrom, A.J., Izraeljan, A., Verseyden, C., Collins, J.M., Frayn, K.N., Plokker, T.W., Elte, J.W. and *C.M. Castro* (2008). Leukocyte activation by triglyceride-rich lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **28**, 792-797.

Appleton, K.M. and *J.E. Blundell* (2007). Habitual high and low consumers of artificially-sweetened beverages. Effects of sweet taste and energy on short-term appetite. *Physiol. Behav.* **92**, 479-486.

Atasayar, S., Gurer-Orhan, H., Orhan, H., Gurel, B., Girgin, G. and *H. Ozgunes* (2009). Preventive effect of aminoguanidine compared to vitamin E and C on cisplatin-induced nephrotoxicity in rats. *Exp. Toxicol. Pathol.* **61**, 23-32.

Blackburn, G. L. (1999). Sweeteners and weight control, In: *Low-Calorie Sweeteners: Present and Future* (Ed. Corti, A.),77-87. Karger, Basel.

Blackburn, G.L., Kanders, B.S., Lavin, P.T., Keller, S.D. and *J. Whatley* (1997). The effect of aspartame as part of a multidisciplinary weight-control program on short- and long-term control of body weight. *Am. J. Clin. Nutr.* **65**, 409-418.

Beck, B., Burlet, A., Max, J.P. and *A. Stricker-Krongrad* (2001). Effects of long-term ingestion of aspartame on hypothalamic neuropeptide Y, plasma leptin and body weight gain and composition. *Physiol. Behav.* **75**, 41-47.

Beutler, E. (1975a). Reduced Glutathione (GSH), In: *Red Cell Metabolism, a Manual of Biochemical Methods* (Ed Beutler, E.), 112-114. Grune and Straton, New York.

Beutler, E. (1975b). Oxidized Glutathione (GSSG), In: *Red Cell Metabolism, a Manual of Biochemical Methods* (Ed Beutler, E.), 115-117. Grune and Straton, New York.

Butchko, H.H., Stargel, W.W., Comer, C.P., Mayhew, D.A., Benninger, C., Blackburn, G.L., Sonneville, L.M., Geha, R.S., Hertelendy, Z., Koestner, A., Leon, A.S., Liepa, G.U., McMartin, K.E., Mendenhall, C.L., Munro, I.C., Novotny, E.J., Renwick, A.G., Schiffman, S.S., Schome, R.D.L., Shaywitz, B.A, Spiers, P.A., Tephly, T.R., Thomas, J.A. and *F.K. Trefz* (2002). Aspartame. Review of safety. *Regul. Toxicol. Pharmacol.* **35**, 1-93.

Caro, J.F., Sinha, M.K., Kolaczynski, J.W., Zhang, P.L. and *R.V. Considine* (1996). Leptin. The tale of an obesity gene. *Diabetes* **45**, 1455-1462.

Castro, G.D., Costantini, M.H., Delgado de layno, A.M. and *A. Castro* (2002). Rat liver microsomal and nuclear activation of methanol to hydroxyl methyl free radicals. *Toxicol. Lett.* **129,** 227-236.

Chaitanya, K.V., Pathan, A.A.K., Mazumdar, S.S., Chakravarthi, G.P., Parine, N. and *V. Bobbarala* (2010). Role of oxidative stress in human health. An overview. *J. Pharm. Res.* **3**, 1330-1333.

Chen, P.E., Geballe, M.T., Stansfeld, P.J., Johnston, A.R., Yuan, H,. Jacob, A.L., Snyder, J.P., Traynelis, S.F. and *D.J. Wyllie* (2005). Structural features of the glutamate-binding site in recombinant NR1/NR2A N-methyl-D-aspartate receptors determined by site-directed mutagenesis and molecular modeling. *Mol. Pharmacol.* **67**, 1470-1484.

Choudhary, A.K. and *R.S. Devi* (2014). Serum biochemical responses under oxidative stress of aspartame in *Wistar albino* rats. *Asian Pac. J. Trop. Dis.* **4**, 403-410.

Das U.N. (2010). Obesity. genes, brain, gut, and environment. *Nutrition* **26**, 459-473.

Dhabhar, F.S. and *B.S. McEwen* (1997). Acute stress enhances while chronic stress suppress cell-mediated immunity in vivo. a potential role for leukocyte trafficking. *Brain. Behav. Immun.* **11**, 286-306.

Evans, M., Khan, N. and *A. Rees* (1999). Diabetic dyslipidaemia and coronary heart disease. new perspectives. *Curr. Opin. Lipidol.* **10**, 387-391.

Feijo, F.M., Ballard, C.R., Foletto, K.C., Batista, B.A.M., Neves, A.M., Ribeiro, M.F.M. and *M.C. Bertoluci* (2013). Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult Wistar rats, at similar total caloric intake levels. *Appetite* **60**, 203-207.

Gibbs, J., Falasco, J.D. and *P.R. McHugh* (1976). Cholecystokinin-decreased food intake in rhesus monkeys. *Am. J. Physiol.* **230**, 15-18.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and *S.R. Tannenbaum* (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal. Biochem.* **126**, 131-138.

Griffith, O.W. (1999). Biologic and pharmacologic regulation of mammalian glutathione
synthesis. *Free Radic. Biol. Med.* **27**, 922-935.

Halliwell, B. and *Gutteridge, J.M.C.* (2007). *Free Radicals in Biology and Medicine* fourth ed., 30-110. Oxford University Press, New York.

Humphries, P., Pretorius, E. and *E.H. Naud* (2008). Direct and indirect cellular effects of aspartame on the brain. *Eur. J. Clin. Nutr.* **62**, 451-462.

Iyyaswamy, A. and *S. Rathinasamy* (2012). Effect of chronic exposure to aspartame on oxidative stress in the brain of albino rats. *J. Biosci.* **37**, 679-688.

Jang, W., Jeoung, N.H. and *K.H. Cho* (2011). Modified apolipoprotein (apo) A-I by artificial sweetener causes severe premature cellular senescence and atherosclerosis with impairment of functional and structural properties of apoA-I in lipid-free and lipid-bound state. *Mol. Cells.* **31,** 461-470.

Kim, J.Z., Seo, J. and *K.H. Cho* (2011). Aspartame-fed zebrafish exhibit acute deaths with swimming defects and saccharin-fed zebrafish have elevation of cholesteryl ester transfer protein activity in hypercholesterolemia. Food Chem. Toxicol. **49**, 2899-2905.

Kissileff, H.R., Pi-Sunyer, F.X., Thornton, J. and *G.P. Smith* (1981). C-terminal octapeptide of cholecystokinin decreases food intake in man. *Am. J. Clin. Nutr.* **34**, 154-160.

Leibowitz, S.F. (1980). Neurochemical systems of the hypothalamus-control of feeding and drinking behaviour and water-electrolyte excretion. In: *Handbook of the Hypothalamus* (Eds. P.J. Morgane, and J. Panksepp), 299-343. Marcel Dekker, New York.

Magnuson, B.A., Burdock, G.A., Doull, J., Kroes, R.M., Marsh, G.M., Pariza, M.W., Spencer, P.S., Waddell, W.J., Walker, R. and *S.G.M. William* (2007). Aspartame. a safety elevation based on current use levels, regulations, and toxicological and epidemiological studies. *Crit. Rev. Toxicol.* **37**, 629-727.

Mourad, I.M. (2011). Effect of aspartame on some oxidative stress parameters in liver and kidney of rats. *Afr. J. Pharm. Pharmacol.* **5**, 678-682.

Ohkawa, H., Okishi, N. and *K. Yagi* (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95** 351-358.

Pandey, K.B. and *S.I. Rizvi* (2010). Markers of oxidative stress in erythrocytes and plasma during aging in humans. *Oxid. Med. Cell. Longev*. **3**, 2-12.

Parthasarathy, J.N., Ramasundaram, S.K., Sundaramahalingam, M. and *S.D. Pathinasamy* (2006). Methanol induced oxidative stress in rat lymphoid organs. *J. Occup.* Health **48**, 20-27.

Shelly, C.Lu. (2009). Regulation of glutathione synthesis. *Mol. Aspec. Med.* **30**, 42-59.

Stanley, B.G., Ha, L.H., Spears, L.C. and *M.G. Dee* (1993). Lateral hypothalamic injections of glutamate, kainic acid, D,L-alpha-amino-3-hydroxy-5-methyl-isoxazole propionic acid or N-methyl-D-aspartic acid rapidly elicit intense transient eating in rats. *Brain Res.* **613**, 88-95.

Swithers, S.E. and *T.L. Davidson* (2008). A role for sweet taste. Calorie predictive relations in energy regulation by rats. *Behav. Neurosci.* **122**, 61-173.

Vijan, S. (2010). "Type 2 diabetes". *Ann. Intern. Med.* **152**, 15-31.

Walton, R.G. (1988). The possible role of aspartame in seizure induction. In: *Dietary Phenylalanine and Brain Function* (Eds. R.J. Wurthman, and E. Ritter-Walker), 159-162. Birkhauser, Boston.

Wurtman, R.J., Heftl, F. and *E. Melamed* (1981). Precursor control of neurotransmitter synthesis. *Pharmacol. Rev.* **32**, 315-335.

Yang, Q. (2010). Gain weight by "going diet?" Artificial sweeteners and the neurobiology of sugar cravings. *Yale J. Biol. Med.* **83**, 101-108.