Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Husbandry and Food Science and Technology, Vol. XXI/A 2022
Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol.XXI/A 2022

STUDY ON ASPARTAM TOXICITY AND INFLUENCE ON LABORATORY ANIMALS

Moiș Raul*, Ciurcean Marius*, Gore Victoria*, Vrabie Andreea*, Andronie Luisa*, Coroian Aurelia*

*University of Agriculture Sciences and Veterinary Medicine, Cluj-Napoca, Faculty of Animal Science and Biotehnology, Mănăștur Str 3-5, 400272, Cluj-Napoca, Romania, e-mail: raul.mois@student.usamvcluj.ro, marius.ciurcean@student.usamvcluj.ro; victoria.gore@student.usamvcluj.ro, andreeamaria.vrabie@student.usamvcluj.ro

*Corresponding author**:** luisa.andronie@usamvcluj.ro, aurelia.coroian@usamvcluj.ro

Abstract

Aspartame is used in very many foods. The maximum permissible dose for humans is 40 mg/kg body weight/day. For a food additive to be placed on the market, it must pass the assessments of the World Health Organisation and several types of studies must be carried out in which it is proved that it is not toxic to the animal and human organism. In these studies, the doses in which it can be used in food and the doses at which it can become toxic should also be established. Aspartame is a chemical, consisting of 3 elements: 50% phenylalanine, 40% aspartic acid and 10% methanol. The purpose of this study is to highlight the effects produced by aspartame on laboratory animals contained in various studies.

Keywords: aspartame, toxicity, metabolism, organ diseases

INTRODUCTION

Food additives are substances that are not normal components of the food itself, but are intentionally added to the food for different purposes, such as: nutritional, technological and organoleptic (Tofană M., 2006). Aspartame is used in many foods, over 6000, but it has some restrictions and maximum doses allowed. The maximum permitted dose for humans is 40 mg/kg bodyweight/ day, so as not to affect human health. In order for a food additive to be placed on the market, it must pass World Health Organization assessments and different types of studies must be carried out to show that it is not toxic to the animal or human body. These studies should also determine the doses at which it can be used in food but also the doses at which it can become toxic. Aspartame was accidentally discovered in 1965 by chemist James Schlatter, who worked for G.D. Searle and was testing an anti-ulcer drug. Aspartame is a chemical product consisting of 3 elements: 50% phenylalanine, 40% aspartic acid and 10% methanol. It also has other names on the market, such as: NutraSweet, Equal, Spoonful, Equal-Measure, all to mislead consumers. The aim of this study is to highlight some of the side effects that aspartame has on laboratory animals

Toxicological aspects of aspartame, its influence on the body

In a study in April 2012 carried out by Kate S. Collison and her team on 3 groups of mice, the way aspartame influenced blood glucose levels, insulin, and sex-based fat deposition was observed. One of the groups was the control group and the other two were given aspartame in water. The results of these parameters differ according to sex, namely: in males the body weight increased significantly compared to the control group but in the females in this experiment there was no significant increase in body weight. In contrast, visceral fat increased more in the experimental groups for both sexes and the blood glucose level increased significantly, especially in males. The conclusions of this study were that feeding aspartame to mice can affect, in addition to blood glucose levels, insulin and adipose tissue deposition, especially in the visceral area, also memory capacity as aspartame, by the nature of its composition, is an excitotoxin (Collison et al., 2012). The carcinogenic effect of aspartame on mice was observed in another experiment conducted by Morando Soffritti and colleagues. Both sexes were given 20 g/day of aspartame. The experiment began when the animals were 8 weeks old and ended when the animals died. The study ended after 151 weeks when the last mouse, who was 159 weeks old, died. Histological and pathological studies were performed after death and it was observed that at the lowest dose of aspartame administered (8 mg/kg), out of 100 individuals, 32 had various malignant tumours and 23 had leukaemia. At the highest dose administered, i.e. 50 mg/kg bodyweight, also out of 100 individuals, 55 had tumours and 29 had leukaemia. In this study it was shown that aspartame has a high carcinogenic potential, even a dose of 20 mg/kg bodyweight being able to cause various malignant and benign tumours, this while the daily dose allowed in Europe is 40 mg/kg bodyweight. Therefore, this study contradicts the results of the FDA-approved experiment in 1981, and these studies, which state that aspartame has no carcinogenic effects on mice and humans (Soffritti et al., 2005), should be re-evaluated.

Ralph G. Walton and Woodrow C. Monte conducted a study regarding the correlation between methanol intake, such as aspartame which contains methanol, and autism. In this study, they interviewed several women who were separated into two groups, namely those who gave birth to children with autism (161 questionnaires) and those who gave birth to healthy children (550 questionnaires completed by them). Based on the questionnaires, the approximate dose of methanol ingested by women per week was calculated. The women who gave birth to healthy children consumed 66.71 mg per week of methanol and those who gave birth to children with autism consumed an average of 142.31 mg methanol/week, so

it is apparent that a high consumption of methanol-containing products can cause various intrauterine malformations, thus there being a very high possibility that the children may be born with autism in addition to other problems that might occur after birth. Methanol is also suspected to cause various birth defects in children whose mothers have been in a methanol environment so, after many studies, it has been concluded that it is responsible for premature births, central nervous system damage, blindness and autism in children (Walton et al., 2015). In a study conducted by Kate S. Collison and her team on the interaction of the effects of aspartame and sodium monoglutamate when new-borns are exposed to these additives, they found that both contain amino acids that can interact with receptors in the brain, thus possibly causing a number of more or less known problems. Sodium monoglutamate consumed in high doses has been proven to cause neuroendocrine dysfunctions in mammals in the neonatal period, and aspartame, although it is a low-caloric sweetener, has been proven to be responsible for raising blood glucose levels, weight gain and not in the least it is responsible for increasing the amount of visceral adipose tissue, which severely affects vital organs. This experiment studied the effect of these two additives, aspartame and sodium monoglutamate, both separately and in combination, on body weight but also on glucose and insulin homeostasis. Aspartame alone, used at a dose of 50 mg/kg bodyweight, increased the blood glucose by 1.6 times compared to the control group who were not given any additives. Monosodium glutamate lowered blood triglycerides and total cholesterol levels. The combination of these additives (120 mg/kg body weight) however led to a significant increase in weight and caused a further 2.3 fold increase in blood glucose, but these two also caused insulin intolerance compared to the control group. The diet of mice with aspartame, sodium monoglutamate or a combination of these two, began in the womb as amino acids crossed the placenta, continued during lactation and then through drinking water. This was the first study to determine the hyperglycaemic effects of chronic exposure of mice to the combination of aspartame and sodium monoglutamate, which are frequently consumed as part of our daily diet. However, in order to correlate the effects of these two additives in vivo, more studies should be performed on this subject in other animal species other than mice. Even if these studies are done over long or short periods of time, or if they are done on mice or other animal species, we can realize what devastating effects these food additives have on the human body (Collison et al., 2012). The heart is another organ affected by this sweetener but it is not given as much importance as the other organs, since the liver, kidneys and nervous system are affected first and foremost. The first organ affected by any kind of alcohol poisoning is the heart and since aspartame contains methanol it’s obvious this organ is also affected.

This substance over time can cause changes in the heart muscle, a disease called cardiomyopathy. Aspartame, or rather aspartame's toxic components, attack the mitochondria in the cells, of which the heart contains the most, destroying mitochondrial DNA. The biggest problem is that mitochondrial DNA passes from one generation to another in women and thus, fetuses, the children of the next generations who do not consume aspartame, will still affected by this problem. It has been found that athletes who consume creatine in combination with aspartame are at risk of sudden death due to discontinuation of heart activity. Some symptoms of this disease are: weakness and fatigue at low exertion, chest pain, fainting, bruising of the skin, enlarged veins, enlarged liver, weight loss and loss of appetite, altered breathing and palpitations. These are just some of the symptoms of aspartame-induced cardiomyopathy (James Bowen., 2003).

Table 1

Studies regarding aspartame toxicity and its symptoms
Clinical aspects of laboratory animals exposed to aspartame

AUTHORS	GROUPS	DOSAGE	SYMPTOMS
Kate S. Collison et al. (2012).	3 batches of mice: 2 experimental and one control.	55.14 mg/kg bodyweight for both experimental groups.	In males, body weight increased significantly compared to the control group, but in females there was no significant weight gain. Blood glucose and visceral fat levels increased compared to the control group, especially in males.
Iyaswamy Ashok., Rathinasamy Sheeldevi (2015).	3 batches of rats, each of 6 animals: 2 experimental and one control.	40 mg/kg bodyweight for both experimental groups.	It affects the activity of liver enzymes, contributes to the creation of free radicals and thus leads to hepatotoxicity.
Eyyüp Rencüzogullari et al. (2004).	4 healthy, non-smoking people each donated 0.2 ml of blood, from which culture media were made on which aspartame was applied.	500, 1000, 2000 µg/ml with a treatment period of 24 and 48 hours.	Aspartame induces an increase in chromosomal aberrations at all concentrations compared to the control group (breakage of chromosomes, union of chromatids), in addition at all concentrations the mitotic index decreases while at the highest concentration the replication index also decreases.
Arbind Kumar Choudhary et al. (2015).	4 groups; group 1: control, group 2: folic acid deficiency, group 3: treated with aspartame 90 days,	40 mg/kg bodyweight for groups 3 and 4.	It acts as a chemical stressor, intervenes in the oxidative balance, dominates the sympathetic innervation on the heart and reduces the action of the vagus nerve on the heart,

	group 4: folic acid deficiency and treated with aspartame 90 days.		decreases the heart rate and affects the automatism.
K. S. RAO et al. (1972).	5 male and 2 female newborn monkeys of the genus Rhesus divided into 3 groups all experimental without control group.	3 different doses: 1 g/kg/ day, 3 g/kg/day and 4-6 g/kg/day aspartame dissolved in milk.	After 218 days of treatment, seizures and epileptic seizures were observed in animals which received medium and high doses; after 300 days an animal died (of unknown causes). Out of 7 animals, 5 suffered from spasmodic seizures and one died.
Iman M. Mourad., Neveen A. Noor (2011)	2 groups of rats: one experimental and one control.	40 mg/kg bodyweight daily administered orally and dissolved in distilled water.	It leads to an increase in oxidative stress in the cerebral cortex, increases the level of nitrates in the blood and stimulates lipid peroxidation.
Arbind, K. C et al. (2014).	5 groups; group 1: control group, group 2: folic acid deficiency raised with MTX solution, group 3: folic acid deficiency and treated with 40 mg/kg bodyweight aspartame for 15 days, group 4: folic acid deficiency and treated with 40 mg/kg for 30 days and group 5: folic acid deficiency and treated with aspartame for 90 days.	40 mg/kg bodyweight for groups 3, 4 and 5.	Inhibits the function of ATPase, alters cell membrane fluidity, excessively increases free radicals that induce oxidative stress in blood cells.
Morando Soffritti et al. (2010).	6 groups of mice and rats of 62-122 males and females	2, 8, 16 and 32 g/kg bodyweight	A very high level of hepatocellular carcinomas was observed in males at the highest doses: 16 and 32 g/kg bodyweight. Alveolar carcinomas also appeared at these doses, so in high doses, aspartame is a carcinogenic agent for at least 2 species of animals.

C. Trocho et al. (1998).	2 groups of mice: one experimental and one control group.	10 mg/kg bodyweight radioactive aspartame to see how much reaches the organs and which ones are most affected.	The highest radioactivity was found in plasma and liver, approximately 98%. The most affected organs were the brain, liver and kidneys.
Morando Soffritti et al. (2007).	2 groups of rats, each of 70-95 males and females.	One group was treated with 2 g/ kg body weight and the other with 40 mg/kg body weight.	A large number of malignant tumours and an increased incidence of leukaemia were observed in males at the dose of 2 g/kg bodyweight. In females treated with the highest dose, a large number of breast cancers were observed.

The way aspartame is assimilated by laboratory animals

At a dose of 50 mg/kg/day (maximum dose accepted in America) and 40 mg/kg/day (maximum dose accepted in Europe), aspartame is not considered to be toxic to animals or humans and it's not believed to cause health problems, but many studies have shown that even at this dose, when consumed daily, the human and animal bodies suffer from the metabolites resulting from aspartame. Methanol, aspartic acid and phenylalanine are the base components of aspartame, of which methanol is by far the most harmful to health because it produces a number of devastating side effects to the body (Ashok and Sheeladevi, 2014). This synthetic sweetener can be found in over 6.000 foods and once in the body, it is metabolized into three components: phenylalanine, aspartic acid and methanol which has the role of binding the other two molecules. Phenylalanine is an essential amino acid without which the human body cannot function properly, and since this amino acid is not produced by the body, it must be procured from food. This compound is needed for the production of certain hormones and neurotransmitters and can be found in many natural products. Phenylalanine acts on the central nervous system, where it can accumulate in too large amounts that are not beneficial to the body. It is converted to tyrosine from which adrenaline, noradrenaline, dopamine and a number of thyroid hormones are formed. High consumption of aspartame causes the formation in abundance of noradrenaline and adrenaline which will increase brain excitability, overexciting nerve cells that will gradually die. This excitotoxin causes certain aggressive states, epileptic seizures, and seizures that may

unfortunately resemble other diseases and so we cannot attribute these problems to aspartame (Lipton E., et al. 1991).

REFERENCES

1. Arbind K.C., Devi S.R., 2014, Role of Antioxidant Enzymes in Oxidative Stress and Immune Response Evaluation of Aspartame in Blood Cells of Wistar Albino Rats, International Food Research Journal 21(6), pp. 2263-2272.
2. Arbind K.C., Sundareswaran L., Devi S.R., 2015, Effects of Aspartame on the Evaluation of Electrophysiological Responses in Wistar Albino Rats. Journal of Taibah University for Science doi: 10. 1016/j.jtusci. 2015.07.006.
3. Ashok I., Sheeladevi R., 2014, Oxidant Stress Evoked Damage in Rats Hepatocytes Leading to Triggered Nitric Oxide Synthase (NOS) Levels of Long Term Consuption of Aspartame. Journal of Food and Drug Analysis, pp. 1-13.
4. Ashok I., Sheeldevi R., 2015, Oxidant Stress Evoked Damage in Rat Hepatocyte Leading to Triggered Nitric Oxide Synthase (NOS) Levels on Long Term Consumption of Aspartame. Journal of Food and Drug Analysis, 23(4), 679-691.
5. Bowen J.M.D., 2003, Cardiomyopathy from Aspartame.
6. Collison K.S., Makhoul N.J., Zaidi M.Z., Al-Rabiah R., Bernard A.I., Rosario L.A., Shoukri U.M., Al-Mohanna F.A., 2012, Interactive Effects of Neonatal Exposure to Monosodium Glutamate and Aspartame on Glucoze Homeostasis. Nutr Metab, 9-58.
7. Collison K.S., Makhoul N.J., Zaidi M.Z., Saleh S.M., Bernard A.A., Al-Rabiah I.R., Al-Mohanna F.A., 2012, Gender Dimorphism in Aspartame-Induced Impairment of Spatial Cognition and Insulin Sensitivity. Plos One 7(4), e31570.
8. Eyyüp R., Tüylü B.A., Mehmet T., Hasan B.I., Kayraldiz A., Arslan M., Diler B.S., 2014, Genotoxicity of Aspartame. Drug and Chemical Toxicology, 27(3), pp. 257-268.
9. Lipton W.E., Yi-Ning L., Kabir Y., Stegink L.D., 1991, Intestinal Absorption of Aspartame Descomposition Products in Adult Rats. Metabolism, 40(12), pp.1337-1345.
10. Mourad I.M., Noor N.A., 2011, Aspartame (a widely used artificial sweetener) and Oxidative Stress in the Rat Cerebral Cortex. Int J Pharm Biomed Sci, 2(1), pp. 4-10.
11. Rao K.S., Mc Connell R.G., Waisman H.A., 1972, SC-18862 52 Week Oral Toxicity Study in the Infant Monkey. Patology -Toxicology.
12. Soffritti M., Belpoggi F., Degli Esposti D., Lambertini L., Tibaldi E., Rigano A., 2005, First Experimental Demonstration of the Multipotential Carcinogenic Effects of Aspartame Administred in the Feed to Sprague-Dawley Rats. Environ Health Perspect, 114(3), 379-385.
13. Soffritti M., Belpoggi F., Esposti D.D., Lambertini L., 2005, Aspartame Induces Lymphomas and Leukaemias in Rats, Eur. J. Oncol, 10(2), pp. 107-116.
14. Soffritti M.F., Belpoggi F.E., Tibaldi D.D., Esposti M., 2007, Life-span Exposure to Low Doses of Aspartame Beginning During Prenatal Life Increases Cancer Effects in Rat, Environ Health Perspect, 115(9), 1293-7.
15. Soffritti, M., Belpoggi F., Tibaldi M.M., Lauriola E., Falcioni M.L., Bua L., 2010, Aspartame Administred in Feed, Beginning Prenatally Through Life Span, Induces Cancers of the Liver and Lung in Male Swiss Mice. Am J Ind Med, 53(12), 1197-206.
16. Tofană M., 2006, Aditivi alimentari-Interacțiunea cu alimentul, Editura Academic pres, Cluj-Napoca.
17. Trocho C., Pardo R., Rafecas I., Virgili J., Remesar X., Fernandez-Lopez J.A., Alemany M., 1998, Formaldehyde Derived From Dietary Aspartame Binds to Tissue Components in Vivo, Barcelona Spain.

18. Walton R.G., 1988, The possible Role of Aspartame in Seizure Induction. In: Dietary Phenylalanine and Brain Function (eds. R. J. Wurthman, Ritter-Walker), pp. 159-162.
19. Walton R.G., Monte W.C., 2015, Dietary Methanol and Autism. Med Hypotheses, 85(4), pp. 441-446.