Hepatotoxic and nephrotoxic effect of acrylamide from potato chips in mice

Mihaela-Claudia SPATARU[1], Ivona POPOVICI[1], Sorin-Aurelian PAŞCA[1], Geta PAVEL[1], Carmen SOLCAN[1]
USAMV Iasi, Faculty of Veterinary Medicine
Corresponding autor: ivona.laiu@yahoo.com

Abstract
Potato chips are considered to be potentially health risk products because they contain several substances with toxic potential effect upon various organs. These snacks contain acrylamide, a multi-organ carcinogenic effect substance and monosodium glutamate, used to improve the taste quality, but which has toxic effects upon several organs. In order to test the effect of potato chips diet upon different organs, two experiment were conducted. In an experiment were used adult mice aged between 4-6 months and in the other young mice of 17-20 days. They were fed for 60 days with potato chips representing 80% of the daily diet. In the first 30 days of experiment the adult mice gain weight, but at the end of the experiment they lost 10-15% from the initial weight measured at the beginning of the experiment. Histopathological modifications were noticed in internal organs of both young and adult mice. Liver presented changes in architecture, necrosis areas, hepatocytes with macrovesicular steatosis, and hydropic degeneration. Into the renal cortex, enlarged glomeruli, mesangial cell proliferation, and reduced urinary spaces were observed along with vascular congestion. Also, in the kidney were noticed renal tubules degeneration, narrow lumens and swelling epithelia. Degenerations were also present in most of intestinal tunics where villi fusion, villi atrophy, modifications in epithelia, in subepithelial connective tissue, and changes in smooth muscle fibers were observed.
Keywords: potato chips, acrylamide, glutamate, multiorgan degeneration, mice

Introduction

Potato chips are familiar foods for children all over the world being well appreciated because their attractive taste and aspect (Konings et al, 2003). Some research reported that potato chips contain high levels of substances that induce neurotoxicity (Abou-Donia et al, 1993; Gad-Allah et al, 2013), genotoxicity (Paulsson et al, 2003; Besaratinia & Pfeifer, 2005; Katen et al, 2016) and carcinogenicity effect (Maronpot et al, 2015) such as acrylamide and some food additives (salt, monosodic glutamate, sugar, etc) which are not present in uncooked food (Exon, 2006; Foot et al, 2007; Riboldi et al, 2014; Sawicka and Mohammed, 2018). Chronic dietary exposure of children was estimated to be on average between 0.5 and 1.9 µg/kg b.w. per day and the 95% was between 1.4 and 3.4 µg/kg b.w. per day (*). In the case of adolescents, adults, elderly and very elderly, the chronic dietary exposure was estimated to be on average between 0.4 and 0.9 µg/kg b.w. per day and the 95% was between 0.6 and 2.0 µg/kg b.w. per day depending on the survey and age group (*). Acrylamide is converted in glycidamide, and Mice are more proficient in doing that compared with either rats or humans (*). In commercial foods that are processed at different temperatures, especially in carbohydrate-rich foods, compounds with cancer risk in humans are formed (Mitka, 2002; El-Sayyad et al, 2011). The acrylamide concentration in fried potato chips ranged from 376 to 2348 microg/kg being in direct correlation with the thermal process and brown coloring (Amrein et al, 2006; Mojska et al, 2008, Friedman & Levin, 2008). The toxic potential of potato chips compounds may produce severe disorders mainly in the liver (Altinoz et al, 2015; Mahmood et al, 2015), kidneys (Mucci et al. 2003, Mucci et al., 2004), heart muscle and cardiovascular system (Naruszewicz et al., 2009), nervos system, and others both in humans and animals.

Material and methods

The study was performed on conventional mice (Mus musculus), 20 individuals, both males and females which were divided into 3 groups: the control one consisted of 4 individuals

and 2 experimental groups of 8 individuals each. In an experiment were used young mice of 17-20 days (first experimental group) and adult mice (the second experimental group) aged between 4-6 months. The experiment lasted for 60 days. During this time, the experimental groups were fed with potato chips, representing 80% of the daily diet, the rest of it consisting of vegetables and cereals. The potato chips come from the distribution network of the local stores, being used several assortments from several producing companies. The control group was fed with vegetables, cereal and mice food. Water was administrated ad libitum and the accommodation to microclimate conditions were ensured in according with national Law no. 43/2014 and to DIRECTIVE 2010/63 / EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The end of the experiment, provided "as without recovery of animals", used the euthanasia of mice according to Art. 16, of Law 43/2014.

Kidney and liver samples were collected from each mouse. Tissue samples from these organs were fixed with Bouin solution, dehydrated with alcohol, cleared with xylene, and paraffin embedded. From each sample 5µm sections were obtained using a Slee microtome. After deparaffination and rehydration the sections were stained with hematoxylin for 10 minutes and eosin for 1 minute. Slides were examined using a Leica microscope and LAS V4.9 soft.

Results

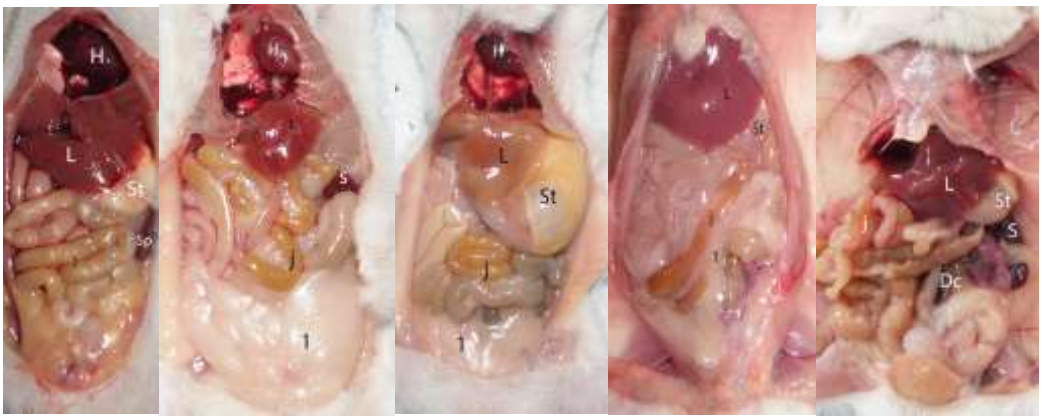
By repeated weight measuring during the experiment, changes in the weight of the mice were found, both in the young mice group (first experimental group) and in adult mice group (second experimental group). At 30 days, in both experimental groups it was observed an increase in weight between 2-4 g in adult mice (approximately 10%) and between 3-6 g in young animals, which represents approximately 15-35% of the initial weight, compared to the control group in which there was registered of approximately 0,5 g.

After 60 days of experiment, there is a reduction in weight between 2-3 g in experimental adults and variable between 0-4 g in youth compared to the initial weight recorded, the weight of control mice increasing during this time by about 1-2 g.

A 30-day necropsy examination found an increase in the volume of mesenteric fat mass in both males and females of young and adult experimental groups (both experimental groups). The stomach and intestines were slightly enlarged and full of food. The liver was intensely colored, in adults it was slightly enlarged in size with evident lobulation (Fig. 1, B, D).

At 60 days of experiment, there was an obvious reduction in the mass of abdominal mesenteric fat in both experimental groups. In 3 of the 4 young mice, the stomach was much dilated with gas compared to the adult stomach, which appeared much smaller in size and predominantly empty (Fig. 1, C, E).

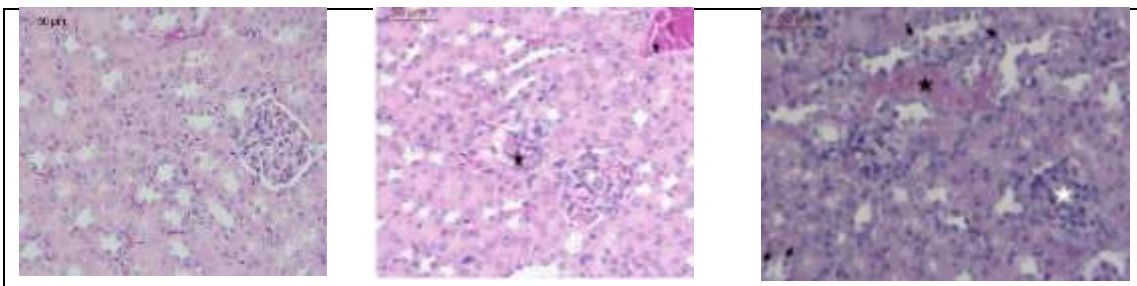
In young mice there was a slight reduction in the size of the liver, which had a light yellowish clay color, without any lobular design, and the gallbladder was rather dilated (Fig. 1, C). In experimental adults, the liver generally appeared intense clay color, the hepatic lobulation being visible through the capsule (Fig. 1, E). On inspection of the kidneys they were intensely colored and surrounded by an obvious fat mass in both experimental groups at 30 days of experiment, after 60 days the kidneys having a lighter color compared to their appearance in the control group, without any visible changes with the naked eye.



A. B. C. D. E.

Fig. 1. The macroscopic aspect of thoraco-abdominal organs in mice used in experiment
A. control mouse, B- experimental young mouse, the 30th days of experiment, C. experimental young mouse, the 60th days of experiment, D- experimental adult mouse, the 30th days of experiment, E. – experimental adult mouse, the 60th days of experiment
Dc- descending colon, J- jejun, H- heart, L- liver, S- spleen, St- stomach, 1- mesenteric mass of fat.

At light microscopy evaluation, the control group kidney samples showed the normal cytoarchitecture (Fig. 2, A). In the kidneys of both experimental group mice, were observed swelling of the renal tubule epithelium, congestion in the glomeruli and between the renal tubules, enlarged glomerulus with reduced urinary space, and hyperplasia of the mesangial cells (Fig 2 B, C). Degeneration of the renal tubule was noticed due to the cloudy swelling of the renal tubule epithelial cells.



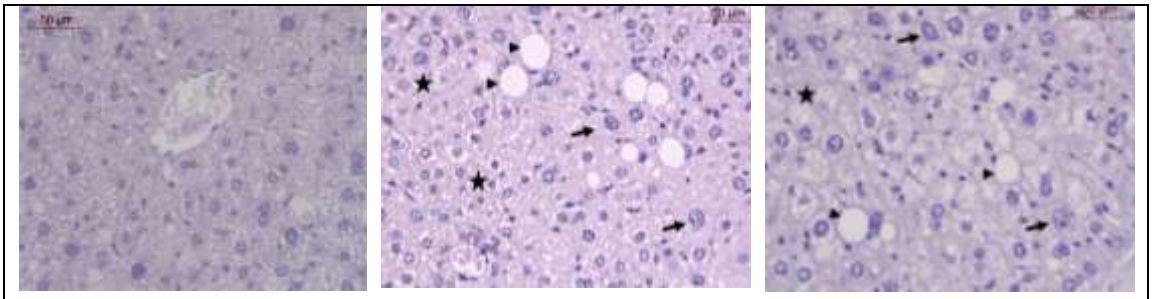
A. B. C.

Fig. 2. Kidney. HE. x400. The histopathological aspects of kidney in mice. Histological normal appearance of kidney (A). Renal congestion (black star) in the glomeruli and between the renal tubules (B, C). Enlarged glomerulus caused by the hyperplasia of mesangial cells (white arrow) (B, C). Degenerated epithelium (arrows) in the renal tubules (C).

Vascular modifications were noticed because of the congestion present between the renal tubules (Fig 2, B, C). Congestion was also noticed in some glomeruli of renal corpuscles in both experimental groups (Fig 2 B, C). The modified renal corpuscles presented enlarged glomerulus with reduced urinary space, caused by hyperplasia of the mesangial cells (Fig 2 B, C).

The liver in control group showed no modifications (Fig 3.A). The liver of both experimental groups presented diffuse areas with severe macro vesicular steatosis, hydropic degeneration, and necrosis. In experimental groups, steatosis areas with hepatocytes presenting

large lipidic vacuoles in the cytoplasm and peripheral nuclei were observed (Fig 3 B, C). Some hepatocytes presented hyperhydration with protidic coagulation in the cytoplasm and large hyperhydrated nuclei (Fig 3 B, C). Necrosis was present in several areas in which hepatocytes with pyknotic nuclei were noticed.



A. B. C.

Fig. 3. Liver. HE, x400. The histopathological aspects of liver in mice. Normal histological appearance of liver (A). Diffuse areas with macro vesicular steatosis (arrowhead) (B, C). Hydropic degeneration (arrow) in hepatocytes presenting enlarged nuclei (B, C). Necrosis (star) present in diffuse areas (B, C).

Discussions

Variations in body weight observed for the mice in our study were described in experiments in which acrylamide was administrated (Sengul et al., 2020).

Vascular congestion in the kidney both in the glomerulus and between the renal tubules was described in several experiments in which acrylamide was administrated to animals (Ghorbel et al., 2014; Sengul et al., 2020). The effect of acrylamide in the kidney structure was observed in the form of tubular degeneration with cytoplasmic hyperoesinophilia, enlarged hyper chromatic nuclei and nucleoli along with hyaline droplets and enlarged Bowman's capsule (Rahej and Al-Daheri, 2017). Degeneration of the renal tubular epithelium with vacuolization, loss of brush borders, rupture of cells, necrosis and hyperemia was described by other authors too (Kandemir et al., 2020; Mahmood et al., 2015). Studies have shown that acrylamide also induces leukocyte infiltration between the tubules and glomerulus fragments (Ghorbel et al., 2016).

Degenerated hepatocytes, with large lipid vacuoles observed in diffuse areas were described by Altinoz et al., 2015. The fatty deposits were observed in hepatocytes with variable size vacuoles, along with chromatolysis and congestion of central veins (Allam et al., 2010). Studies showed liver modifications such as congested blood vessels and degenerated hepatocytes, mostly suffering from necrosis, and mononuclear inflammatory cells infiltration in portal areas (AL-Mosaibih, 2013; Mahmood et al, 2015).

The damaging effect of acrylamide upon both the liver and kidney was showed in several experiments (Kandemir et al., 2020; Mahmood et al., 2015). The exposure to acrylamide increases the generation of free radicals and hydroperoxides which leads to lipid peroxidation (Prasad and Muralidhara, 2012). About 50% of ingested acrylamide is metabolized in liver. The cytochrome P450 biotransforms it into glycidamide, and both are conjugated with glutathione by enzymes from the family of glutathione S-transferase (Tareke et al., 2008). The reaction with glutathione makes acrylamide and its derivatives easily to be eliminated from the organism by excretion in urine (Friedman et al., 2003). Glutathione is a cell antioxidant, but large quantities of ingested acrylamide induces higher activity of antioxidative system and exposure for long time periods induce symptoms of oxidative stress (Semla et al., 2017). Oxidation of biological molecules (lipids,

enzymes, DNA) leads to damage of organelles, impaired cell metabolism, DNA fragmentation and cell death (Greń, 2013). These microscopical changes are translated into clinical symptoms of numerous diseases including diabetes, neurodegeneration, diseases of cardiovascular system (Rahman et al. 2012,). The oxidative stress induced by acrylamide explains the modifications observed in both kidney and liver collected from mice fed with potato chips in our experiment.

Conclusions

Modifications induced by acrylamide were observed in the bodyweight variations and macroscopic and histologic aspects. Degenerations observed in liver and kidney of mice feed with potato chips correspond to the hepatotoxic and nephrotoxic effect of acrylamide.

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