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Research Article

Impairment of Hepatic, Cardiac and Lung Tissues in Aspartame Treated Male Wistar Albino Rats

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Abstract: Aspartame is widely used artificial sweeter as well as in foods products such as soft drinks, hot chocolate, candy, as well as some vitamins and sugar-free cough drops. There is no available work concerned hepato-,cardio-and lung toxicities. The present studies focused on analysis the aspartame associated alterations of the mentioned organs. Fourteen male albino Wistar albino rats weighing approximately 100 gram body weight. They were divided into two main groups. Control and aspartame-treated groups (80mg/kg body weight every other day for 4 weeks). At the end of treatments, animals were sacrificed and liver,heart and lung were dissected and processed for histological investigation and immunohistochemistry of caspase 3. The present findings showed focal collection of inflammatory cells associated with damaging liver, myocardium and alveolar cells. Interstitial fibrosis of the alveoli was detected. Immunohistochemistry of caspase 3 revealed increased increased immunostaining manifesting cell death. Finally the authors concluded that aspartame-treatment led to cardio-,hepato and lung toxicity that must be controlled during administration.

Keywords: soft drinks, hot chocolate, candy, vitamins and sugar-free cough drops,Control and aspartame-treated groups, increased immunostaining manifesting cell death, cardio-,hepato and lung toxicity.

INTRODUCTION

Aspartame is artificial nonnutritive sweeteners, approved by American Food and Drug Administration (FDA. 2009). Its global production reached more than 16 000 tons per year (Belpoggi *et al.,* 2006).In foods and beverages, aspartame (L-aspartyl L-phenylalanine methyl ester) is commonly used (Butchko *et al.,* 2002). In the intestinal lumen, it is absorbed and degraded to phenylalanine (50%); aspartic acid (40%); and methanol (10%).Methanol is further oxidized to formaldehyde and formic acid (Rannry *et al.*, 1976). High levels of methanol were observed post aspartame administration to humans (Davoli *et al.* 1986) and rats (Iyyaswamy & Rathinasamy, 2012 & Abhilash *et al.*, 2011). Formaldehyde is converted to formate via formaldehyde dehydrogenase (Harris *et al.,* 2003). Administration of aspartame has been associated with the development of hyperglycemia (Collison *et al.*, 2012), hepatocellular lesions (Abhilash *et al.,* 2011), pulmonary hypertension (Roberts, 2004), myocardial damage (Choudhary *et al.,* 2016) and nephrotoxicity (Otman & Bin-Jumah ,2019) due to liberation of metabolites, including formaldehyde and format.

However, little studies were concerned with the histopathological effects on liver, kidney, heart and lung. The present study is concerned with illustrating the histopathological and immunohistochemistry of caspase 3 in liver, heart, kidney and lung of breast feeding rats.

MATERIAL AND METHODS

Experimental work:

Fourteen male albino Wistar rats (Rattus norvegicus) weighing approximately 100 g body weight, obtained from, Ministry of Health Farm, Egypt and used to research.

They were placed in an airy space with a light and dark period of nearly 12 hours. The rats were arranged into two groups (n = 7 per each); control and aspartame-treatment. Free access of diet and water were allowed *ad Libitum*. Aspartame was orally administered every other day by dose 80mg / kg body weight for 4 weeks. At the end of treatment, the animals were anaesthetized and sacrificed by cervical dislocation. Liver, kidney, heart and lung were dissected and

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immediately fixed in 10% phosphate buffered formalin (pH 7.4). The specimens were dehydrated in ascending ethyl alcohol, cleared in toluene and mounted in molten paraplast 58-62C^0. Five µm histological sections were carried out and stained with hematoxylin and eosin. For immunohistochemical reaction, the dewaxed tissue sections were digested with 0.05 % trypsin (pH 7.8) and incubated with the caspase 3 antibody(dilution 1:100 Thermo Fisher Scientific, Fremont, CA, USA; Cat. No. A1–70007) for overnight, followed by treatment with a horseradish peroxidase streptavidin detection system (Dako), and DAB for developing the immunostaining and counterstained with hematoxylin. Negative control was carried out by using 1% non-immune serum phosphate buffer solution (PBS) solution. The specimens were investigated under a Leica BM5000 microscope (Leica Microsystems, Wetzlar, Germany) and photographed. Image analysis was carried out of the slides after photographed using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 40 X objective. The result images were analyzed by using Intel® Core I5® based computer using Video Test morphology® software (Russia) and % area measurement was carried out.

RESULTS:

Liver:

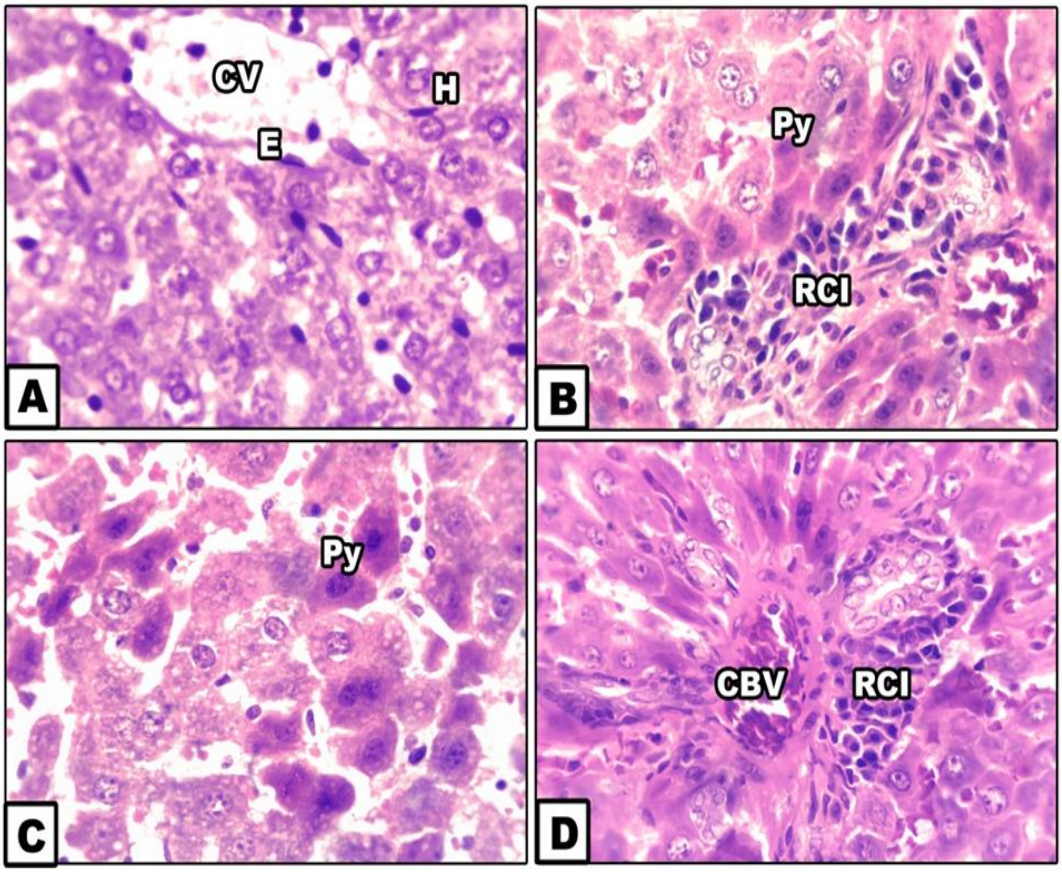

Fig. 1. Photomicrographs of histological section of liver of male rats. A.Control showing hepatic cord radiating from central vein. B. Aspartame-treated liver showing focal collection of inflammatory cells and eosinophilic hepatocytes with pyknotic nuclei. B. Aspartame-treatment showing dilated blood sinuses and eosinophilic hepatocytes with pyknotic nuclei. D. Aspartame-treatment showing congested blood vessel and perivascular round cell infiltration. HE.Abbreviations; CV, central vein; CBV, central blood vessel; E, endothelium; H, hepatocyte; Py, pyknosis; RCI, round cell infiltration.

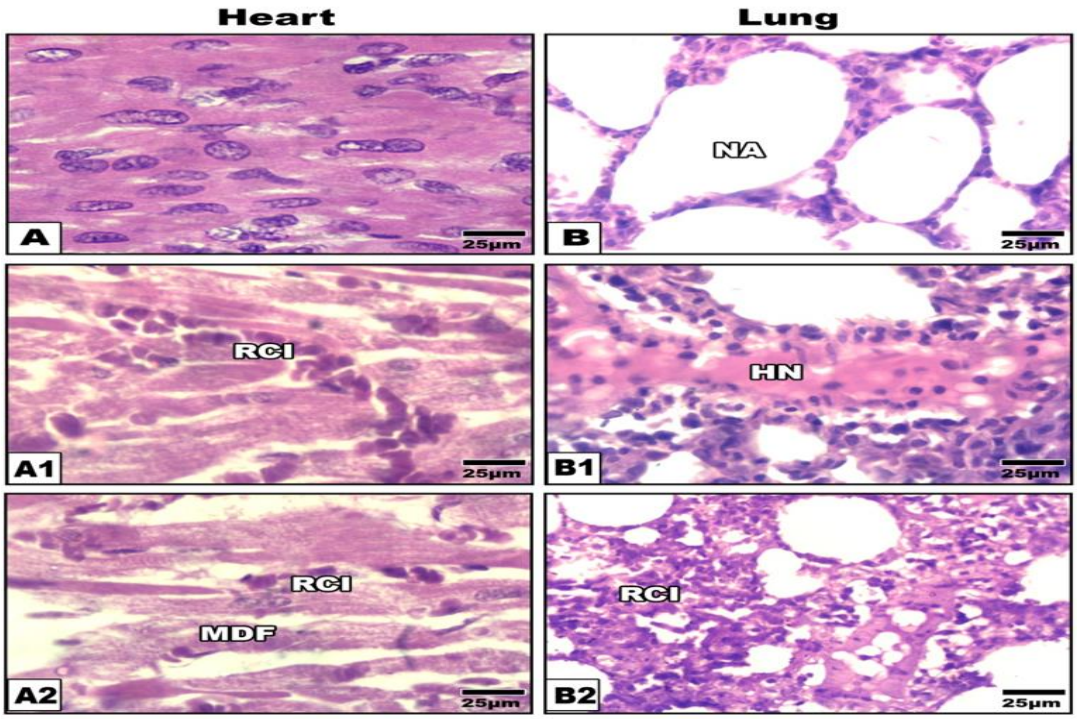

Fig. 2. Photomicrographs of histological section of heart (A-A2) and lung (B-B2) of male rat. A. Control heart showing regular oriented myocardial muscle fiber. A1& A2. Aspartame-treatment showing fragility of muscle fiber with round cell infiltration. B. control lung with normal alveoli of varying sizes lined by alveolar epithelium. B1. Aspartame-treatment showing congested alveoli with round cell infiltration and hyaline necrosis. B2, Aspartame-treatment showing dense collection of inflammatory cells with reduced alveolar lumina. HE. Abbreviations; HN, hyaline necrosis; MDF, massive damaged fiber, NA, normal alveoli; RCI, round cell infiltration.

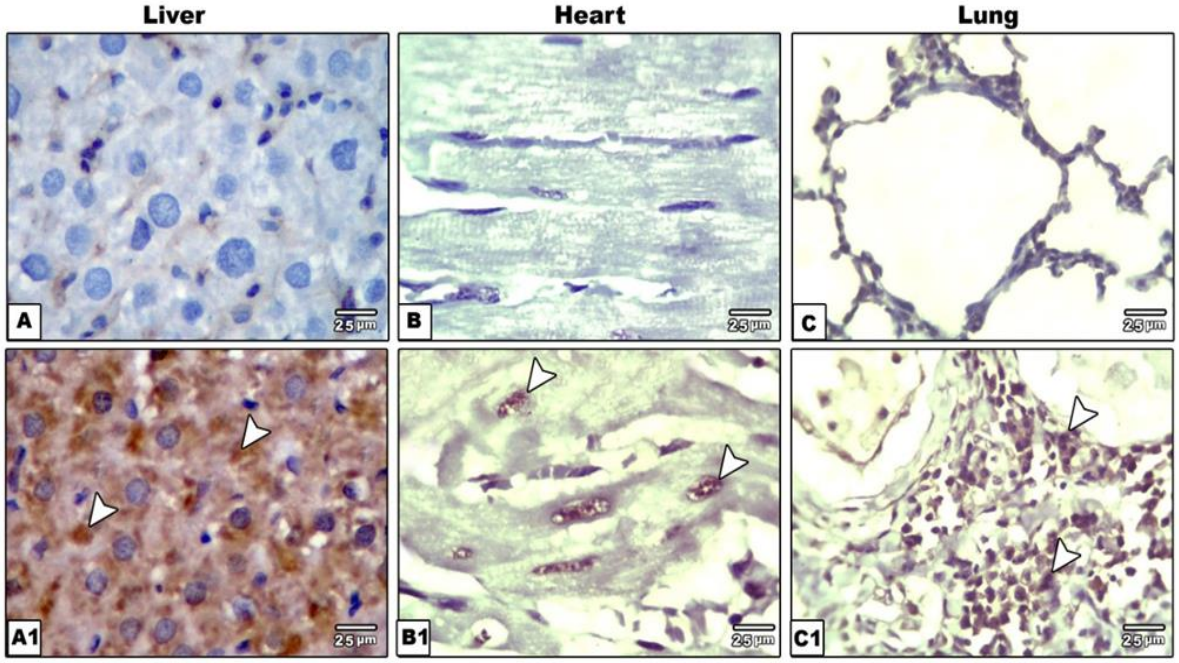

Fig.3. Photomicrographs of formalin fixed histological sections immunohistochemically stained with the antibody caspase 3. A. Control liver showing negative immunostaining. A1. Aspartame-treatment showing increased dark brown reaction of caspase 3. B. Control heart showing negative reaction. B1. Aspartame treated heart showing scattered dense caspase reaction. C. Control lung showing negative reaction. C1. aspartame-treatment showing dark-brown dense reaction in the interstitial cells.Arrow head indicate the positive reaction.

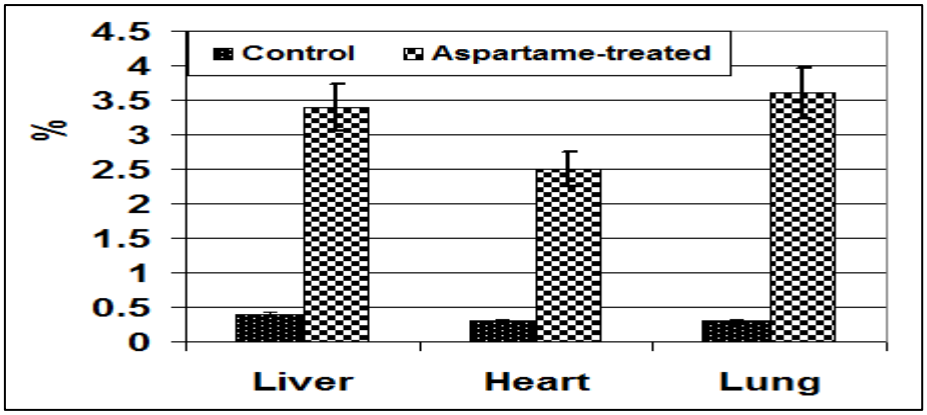

Fig.3. Image analysis of surface reacted area showing significant increase of immunohistochemical reaction of caspase 3 in aspartame-treated liver, heart and lung compared to the control.

In control, the liver is composed of numerous hepatic cords radiating from the central vein and separated from each other by hepatic sinusoids. The hepatic cords are composed of one cell thick. Mono-or bi-nucleated hepatocytes are detected. The blood sinusoids are lined with endothelial cells and enclosed by darker Von Kupffer cells (Fig.1A).

In experimental aspartame-treated group, the hepatic cords lacked normal orientation of hepatic cord and become disorganized. Most of the blood sinusoids became dilated. Many of the cytoplasm of the hepatocytes showed abundant vacuoles of varying sizes. Damaged hepatocytes appeared with eosinophilic cytoplasm and pyknotic nuclei.The nuclear chromatinbecome either karyolysed, clumping or pyknotic. The hepatocytes of the peripheral parenchymatous margin were highly affected explained by the presence of necrotic hepatocytes and dense collection of inflammatory cells. Hypertrophied Kupffer cells were detected. Congested blood vessel and focal dense collection of inflammatory cells were detected (Fig.1 B-D).

Following caspase 3 Immunohistochemistry, aspartame-treatment increased cytoplasmic immunostaing compared to the control (Fig.3 A&A1). Image analysis revealed increased caspase staining affinity compared to the control (Fig. 4).

Heart:

In control, the myocardium is composed of branching and anastomosing of muscle fibers with regularly oriented equal diameters. The sarcoplasm appeared eosinophilic with centrally elongated flattened nuclei. The muscle fibers are connected with each other by intercalated disc (Fig.2 A). However, aspartame-treatment increased the fragility and swollen of the muscle fibers. Focal round cell infiltration was reported in between muscle fibers. Numerous focal necrotic fibers associated with disorganization , fragmentation, sarcoplasmic vacuolation and pyknosis of the nuclei were observed (Fig. 2 A1-A2). Increased immunohistochemical reaction was reported post caspase 3 application (Fig. 3 B-B1). Compared to the control, image analysis revealed increased intensity of caspase 3 (Fig. 4).

Lung:

In control, the lung is composed of alveoli of varying sizes. Their walls are composed of one-two cell layer thick with a considerably thinning and possessed two types of pneumocytes; type I & II in alveolar wall (Fig. 2 B).

In aspartame-treated rats, there was a detected dense collection of inflammatory cells through the peri- and inner of the alveolar space losing their luminal space. There was a detected hyaline necrosis in the interstitial tissue of the lung (Fig.2 B1-B2). Increased caspase 3 imunohistochemistry was detected in the lung tissues of the perialveolar space (Fig.3 C-C1). Compared to the control, image analysis revealed increased intensity of caspase 3 (Fig. 4).

DISCUSSION

The present findings revealed that aspartame induced cardiotoxicity illustrated by disorganization of muscle fibers and dense collection of inflammatory cells in necrotic zone. These was assessed by over expression of caspase 3, the marker of cells death.

These findings are consistent with the work of Al-Eisa *et al.*, (2018) and Choudhary & Devi (2015) however their findings restricted only on disarry of myocardial fibers and detached cardiomyocyte nuclei.

Al-Elisa *et al.*, (2018) explained the aspartame cardiotoxicity by its induction of oxidative stress and increase lipid peroxidation.

The increased expression of caspase 3 in cardiomycytes reflected cell death which supported the work of Findikli and Turkoglu (2014) who reported that

the artificial sweeteners induced genotoxicity. DNA damage may be attributed to the generation of ROS which caused DNA strand breaks and consequently DNA replication (Lin *et al.,* 2007).

Also, aspartame-treatmentshowed hepatotoxicity explained by hepatic necrosis with dense collection of inflammatory cells and congestion of blood vessels associated with increased cell death assessed by increased caspase 3 immunohistochemistry.

The present findings supported the work of Ebraheim and Metwally (2016) and Moubarza *et al.,* (2018) following aspartame –treating animal model. The authors explained hepatotoxicity by altering hepatocytes and liver transaminases.

In addition, aspartame-treatment induced congestion of the lung associated with increased inflammatory cells on both alveolar wall and interstitium losing the alveolar lumina, These histopathological changes were associated with hyaline necrosis and overexpression of capase 3 manifesting cell death.

The present findings supported the work of Roberts (Abhilash *et al.,* 2011) who reported increase pulmonary hypertension in 27-year-old woman consumed aspartame. This led the authors to reported it as aspartame disease.

Myocardial damage reflected pulmonary fibrosis. The inflamed lung tissues facilitated accumulation of extracellular matrix components, particularly collagen, at the site of injury (Agrawal *et al.,* 2016). At the same time acute and chronic lung inflammation is influenced in the development of cardiovascular disease (van der Putten, 2019).

The detected cytotoxicity of aspartame in liver, heart and lung explained the work of Paolini *et al.,* (2017) who reported that aspartame induced carcinogenicity in rat model.

The toxicity of aspartame developed from the cleavage of its main components into aspartic acid and methanol which subsequently oxidized into formaldehyde and formic acid (Rannry *et al*., 1976). The level of methanol were markedly increased post aspartame consumption to humans (Davoli, *et al.,* 1986) and rats (Iyyaswamy & Rathinasamy, 2012; Abhilash *et al.,* 2011).

CONCLUSION:

Finally the authors concluded that aspartame-treatment led to cardio-,hepato and lung toxicity that must be controlled during administration. The cytotoxicity of this artificial sweeter come from their metabolites such as methyl alcohol which degraded to formaldhyde.

Conflict of Interest:
The authors declare that there is no conflict of interest

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