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Research Article
Histopathological Effect of Aspartame on Liver and Kidney of Mice

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Abstract

Background and Objective: Aspartame has been widely used in many low-calorie, non-weight-bearing dietary alternatives, particularly in strategies of physical fitness and health with no or little data about its side effects on human health. Thus in this study, the toxicological effects of aspartame on both the liver and kidneys of female rats were investigated. **Methodology:** Cytotoxicity of aspartame was investigated histologically by using hematoxylin and eosin (H and E) stains. The animals received aspartame in drinking water at a dose of 500 mg kg^{-1} body for a month. Both liver and kidney tissues were subjected for histological and morphological analysis. **Results:** In female rats treated with a dose of aspartame of 500 mg kg^{-1} daily in drinking water a clear toxicological effects on the hepatic tissue were significantly obtained. Significant signs of apoptotic cellular death (necrosis) and intensified of chromatin nuclear condensation with nuclear fragmentation were observed in liver tissue cells following treatment with aspartame. In addition, bleeding, infiltrations, invasion of inflammatory cells and blood bleeding in the inner lining of the central veins of the liver were observed with severe congestion and bleeding in the liver sinuses. Regarding to the kidney tissues, treated with aspartame, shrinkage and disappearance in renal pellets in addition to apoptosis were recorded in kidney tissues compared to control ones. Destruction and bleeding in most renal tissues due to congestion in renal tubules were seen in kidney tissues following treatment with aspartame. **Conclusion:** Although aspartame may have a positive effect in obesity as low-calorie, non-weight-bearing dietary alternatives, histological analysis proved that it produces severe cellular toxicity especially in liver and kidney. The toxicological effects performed mechanically via inflammatory and apoptotic pathways. The data recommend that using of aspartame should be taken under medical supervision along with natural antioxidants.

Key words: Aspartame, cellular toxicity, oxidative stress, free radicals, histology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

People are increasingly interested in fitness and physical health and so many low-calorie, non-weight-bearing dietary alternatives have been sought, including aspartame[1,2].

Aspartame is made up of several elements, aspartic acid, phenylalanine and a little methanol. The aspartame compound is used to desalinate more than 6,000 products consumed by hundreds of millions of people, such as moisturizers, chewing gum, chocolate; sweets, yoghurt, some medicines and non-sugar vitamins; It is also used as anti-inflammatory and anti-fungal agent[3,4]. Daily consumption of 2-3 mg kg^{-1} b.wt./day, which is higher in children and pregnant women and the use of aspartame for the substitution of food products and non-sugar medicines is that the compound is 500 times sweeter than sugar, the risk of this substance is especially for children and pregnant women who take these food products[5].

Aspartame in the gastrointestinal tract is also metabolized into its toxic components, aspartic acid, phenylamine and methanol, in research on rodents and humans[6]. It has been found recently that aspartame is paid. Several studies have confirmed that aspartame is a multipotential carcinogen and increases the risk of lymphoma, leukemia, urinary tract tumors and neurological tumors, even in a daily dose (20 mg kg^{-1}) that is much lower than (40 mg kg^{-1})[6,7]. In addition, several studies have indicated a relationship between aspartame consumption and the risk of type 2 diabetes[8], premature birth[9], cellular toxicity, genetic toxicity and induction of pathological anatomical changes in the DD parotid salivary[10-12]. The researchers noted that aspartame leads to the occurrence of cancers, such as white blood cell cancer, leukemia and cancer of the lymph nodes[13-15].

Previously, aspartame is used in the desalination of soft drinks and is one of the sugar substitutes for diabetics and in production of an anti-ulcer drugs[16-18]. However; several studies indicated that aspartame as food additives may cause cancer in mice[19-23].

It was reported that aspartame intake for 90 days produces severe liver toxicity as it decrease the level of antioxidant capacity such as the depletion of glutathione in the liver[18,22]. Also, it was observed that aspartame in a dose of 250 mg kg^{-1}/day when applied orally for 9 weeks led to an increase in liver and brain weight[24-26].

Histologically, aspartame was shown to produce congestion of hepatic cells around the central vein was observed with expansion of the blood pockets, necrosis of some hepatic cells, emphysema, the appearance of gaps in the liver cell cytoplasm, bile duct hypertrophy and the appearance of fibrosis around the vesicles[24-28]. Aspartame consumption leads to ulcers in liver tissue and alterations of the hereditary system in the liver and bone marrow from white mother mice and their offspring[29-30]. Also, in experimental models, the results suggested that long-term consumption of aspartame leads to an imbalance in the development of antioxidants in the brain[1-6]. Most studied, concluded that long-term use of aspartame is responsible for oxidation and renal-hepatic toxicity[6-8]. From aforementioned studies, although aspartame was included in different foods and drinks as additive and in drug synthesis, however prolonged use of it causes severe cellular changes which may resulted in cancer or severe diseases. Thus in consistent with other previous studies that reveals the toxicological effects of aspartame. Whereas, the toxicological changes were directly proportional to the duration of aspartame administration, doses and improvement after its withdrawal[1,6,13,31]. This current study, trying to examine or give more lights on the cellular toxicological effects of aspartame at doses of 500 mg/day which rarely studied previously especially on the liver and kidneys of female rats.

MATERIALS AND METHODS

Materials: In this experiment, female adult albino mice were used. It was obtained from the Animal House of the Faculty of Pharmacy, King Saud University, Riyadh. The animals were distributed in special cages equipped with drinking water in their ventilated rooms subject to the appropriate natural factors of moisture, light and temperature between 25 and 35°C.

Feeding: The animals were given the appropriate food, which is the feed of animals (No. 648) obtained from the General Organization of Silos and Flour Mills in Riyadh. The experimental animals were selected with an average age of 12-15 weeks and an average body weight of 50-60 g.

Aspartame uses: Aspartame was used and obtained from one of the commercial centers for the sale of foodstuffs and the body was white powder packed in bags of each of 0.8 g was dissolved in drinking water and was placed in drinking water for female rats with a dose of 500 mg kg^{-1} b.wt./day for one month.

Experimental design: Experimental animals were divided into 2 groups; Group 1 (control group, n = 5), these animals were given normal drinking water and Group 2 (Aspartame

treated group, n = 15), the animals received aspartame in drinking water at a dose of 500 mg kg^{-1} body for a month. In all groups, animals were anesthetized and then dissected, liver and kidney samples extracted and placed in the stabilization solutions used in the study.

Assessment of histological analysis: All samples of the liver and kidney were taken from pregnant mice and kept in a 10% neutral formalin solution for the preparation of histological sections using Hematoxylin and eosin (H and E) stains.

RESULTS

Histological analysis

Histological changes in liver structure: Sections of mice livers were analyzed histologically by using Hematoxylin and eosin (H and E) stains. Normal liver sections showed normal liver architecture. The liver of mice consists of several liver lobules confined by the connective tissue and the bovine region. Each hepatic placenta consists of a central vein surrounded by ribbons of interstitial and interconnected hepatic cells forming a complex network that encloses the hepatic sinusoids. Multi polygon hepatic cells appear with a granular cytoplasm containing a spherical nucleus which appears with one or more nuclei as shown in Fig. 1a and b.

The effect of aspartame was evident on the hepatic tissue, where tissue degradation and change in the structure of liver cells showed signs of cellular death (necrosis). In addition, there was a marked deterioration of the internal lining of the liver veins. The concentration of nuclear chromatin and nucleic degradation was observed in most liver cells, endo-thelial lining rupture with congestion in the central vein, narrowing of most of the hepatic pockets, presence of hemolysis and the emergence of multiple cavities within the cells. This indicated the accumulation of lipid droplets, which indicates the onset of liver lipid, invasion of inflammatory cells in some areas around the central vein (Fig. 1c-f).

Histological changes in kidney structure: In normal group, histological sections showed that the kidney is made up of two cortex cortices and appears granular because it contains renal pellets, renal tubules, medulla, the universal tubules and henal hoops. The kidney is made up of the Bowman's capsule, which consists of an outer mantle layer and an inner mantle that surrounds a mass of blood capillaries known as glomerulus. The renal tubules are characterized by proximal close-edged tubules with a narrow cavity lined with low epithelial cells based on a basement membrane. The brush edge, a spherical core nucleus and an acid cytoplasm and distant spores have a large cavity and are characterized by cubic epithelial cells based on a basal membrane with acidic cytoplasm and a large spherical nucleus (Fig. 2a, b).

In aspartame treated group, the effect of the treatment with aspartame was evident on the kidney tissue where severe destruction was observed in the kidney tissue, which resulted in the death and decomposition of most cells in addition to

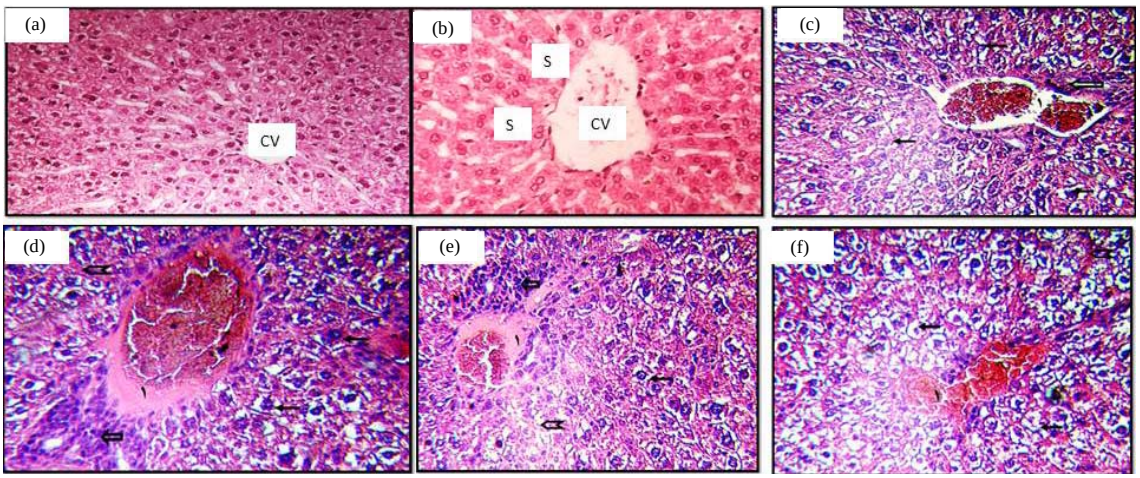

Fig. 1(a-f): Normal and aspartame treated liver tissues, (a-b) Normal structure of the liver tissues with normal central vein (CV) surrounded by ribbons of hepatic cells and the intertwined and covalent (C) confines among themselves the sinuses (s) and (c-f) In aspartame treated liver tissues, showed congestion and bleeding in central venous with death of most liver cells (arrow), invasion of inflammatory cells, infiltrations around the central vein and bleeding and enlargement of the hepatic sinuses (Arrows)

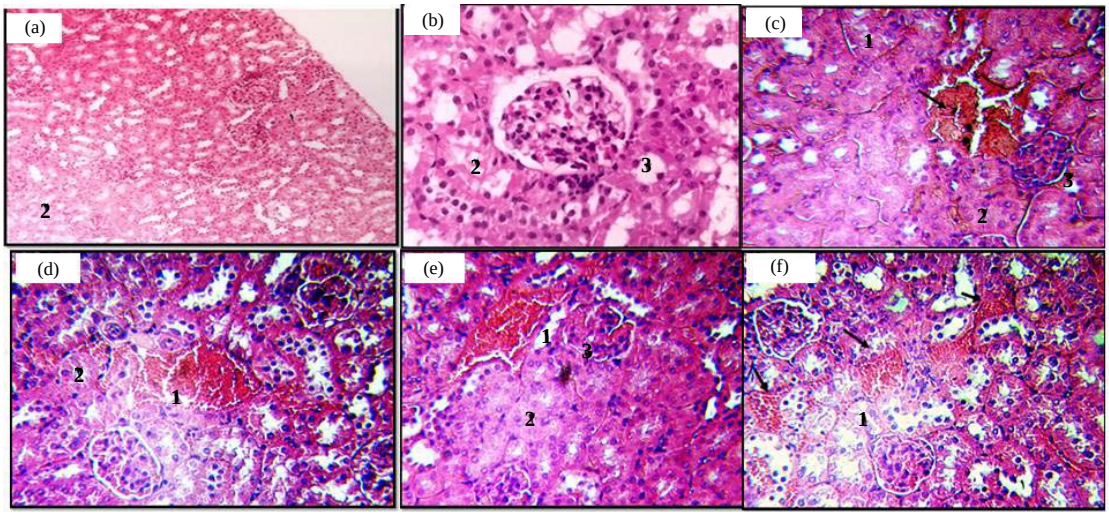

Fig. 2(a-f): Normal and aspartame treated kidney tissues. (a-b) In normal tissues, showed normal the renal cortex, a medullary rays which are a collection of renal tubules that drain into a single collecting duct (1) that drain into a single collecting duct they are formed of close (2) and distant (3) convoluted renal tubules. (c-f) In aspartame treated rats, kidney tissues showed bleeding and degeneration in tubular lining epithelium (arrows), death with focal inflammatory cells infiltration in between the tubules and blood vessels (1) and severe congestion in stromal cortical blood vessels and glomerular tuft (3). Also, death with focal inflammatory cells infiltration in between the tubules and blood vessels also severe congestion and disintegrations in stromal cortical blood vessels and glomerular tuft (1)

the intensification of nuclear chromatin in others, shrinkage and disappearance in the renal globules and the appearance of hemorrhage and narrowness within the glomerular cavity. The destruction of the walls of the renal tubules and their blood congestion, resulting in large areas of destruction and visible bleeding in some regions of the renal tissue, leaving only the remains of some cells (Fig. 2c-f).

DISCUSSION

Today's interest in fitness and physical health has increased and so many low-calorie, cellular toxicity, non-weight-bearing dietary alternatives have been sought, including aspartame[1-3].

In this study, the animals treated with aspartame at doses of 500 mg/day for one month where it is rarely studied in previously reported researches[6,13,25,31]. The effect of aspartame was evident on the hepatic tissue, where tissue degradation and change in structure of liver cells showed signs of cellular death (necrosis). In addition, there was a marked deterioration of the internal lining of the liver veins. Endothelial lining rupture of congestion, dilatation, congestion, dilatation and hemolysis. Invasion of inflammatory cells was observed in some areas around the central vein. The effect of aspartame treatment was evident on the kidney tissue where's the collapse of the renal cavity of the renal glands, the appearance of bleeding and narrowing inside the glomerular cavity, as was observed in the walls of the renal tubules and congestion of blood, which led to the emergence areas of extensive destruction and visible bleeding in some areas of the renal tissue where only the remains of some cells existed. After eating, aspartame decomposed to the remaining natural components; these components include aspartic acid, phenylalanine and methanol. Other decomposing products included formaldehyde, formic acid and duo quito piperazine. High levels of natural essential amino acid (phenylalanine) are a health risk for those born with PKK, a rare genetic disease that prevented vinyl alanine metabolism from occurring properly[5-9].

Previous studies showed that application of aspartame for 90 days or 9 weeks at a dose of dose of 250 mg kg^{-1}/day significantly led to an increase in liver and brain weight. It also increased the level of oxidation in the kidneys, liver, brain and elevated total cholesterol level and triglycerides. Minor lesions were also observed in the brains of rats given aspartame. There were clear infections in the liver tissue where aspartame caused severe necrosis of cells infiltrating the vasculature and expansion of the blood pockets and accumulation of liver

cells. It also resulted in the accumulation of cortex and marrow cells as well as the enlargement of cubic cells in kidney tissues[24-26].

The use of aspartame with a dose of 250 mg kg^{-1} b.wt. and a dose of 1000 mg kg^{-1} b.wt., for 7 weeks resulted in an imbalance in the antioxidant status of the liver tissue resulting from aspartame induced free radicals to reproduce significantly, affecting cellular cytotoxicity. The effect of aspartame was evident on the liver tissue, where congestion of hepatic cells around the central vein was observed with expansion of the blood pockets, necrosis of some hepatic cells, emphysema, the appearance of gaps in the liver cell cytoplasm, bile duct hypertrophy and the appearance of fibrosis around the vesicles[6,28-32].

Histological and genetic structures were shown to be changed following aspartame intake, female white rats and 180 offspring treated with aspartame (50.4 mg). The results revealed a decrease in body weight in mice and young offspring's in all mice of treated groups, histopathological changes and an increase in chromosomal abnormalities and DNA damage were recoreded[6-13]. The results showed that aspartame consumption leads to ulcers in the liver tissue and alterations of the liver and bone marrow system from the white mother mice and their offspring. The mechanism by which aspartame produces cellular toxicity was through oxidative stress pathways, which resulting in initiation of severe toxic free radicals and decreases in antioxidant capacity of the cells via decline in cellular antioxidant enzymes[12-19].

Consistent to present results, many studies have shown that aspartame metabolism in the body generates many toxic substances during metabolism and methanol is the most important. The results of this study indicated that there was a significant reduction in glutathione (GSH), reduced glutathione (GR) side by side with significant increase in lipid peroxidation (LPO), glutathione-S-transfrease (GST), glutamyl peptide transporter (GT) and carbonate and formate level, indicating changes in the development of antioxidants in the liver and brain. There have also been many pathological changes in the liver and renal cortex, which may be methanol itself and its components responsible for changes in the development of antioxidants and histological changes in the liver and renal cortex. Thus, it can be concluded that long-term use of aspartame is responsible for oxidation and renal hepatic toxicity[21-28].

In present study, toxic effects of aspartame to renal cortex, nephrons and kidney structures were significantly evident and may be occurred via both oxidative and apoptotic pathways. Similar to previous studies, these changes can contribute to increased free-radical production which can cause damage to the cell membrane through the peroxidation of unsaturated fatty acids in phospholipids in the cell membrane, accompanied by a change in properties structural and functional membranes[31-32]. Aspartame can cause damage to basic cellular components such as DNA lesions, genetic damage and gene repair activity, leading to cell death due to prolapse and apoptosis[1,6,13,24-37].

The depletion or reduction of antioxidants can contribute to oxidative stress caused by an imbalance in antioxidants. It is believed that oxidative stress plays an important role in the injury of liver and kidney cells. It was reported that, metabolism of aspartame leads to the generation of many free radicals, such as nitrogen and types of oxygen; these free radicals have been shown to damage cellular proteins and DNA. Also, mitochondrial damage was observed which leads to increased membrane permeability and a disturbance in the concentration of ions in the cytoplasm and organelles. This damage was specifically followed by increased permeability of the plasma membrane to sodium, which exceeds the capacity of the pump to extrude sodium and produced the swelling of the cytoplasm due to water buildup, thus showing granules in the cytoplasm and finally causes cellular damage and apoptosis[13,31-40]. Consistent to other studies[1,6,13,29-33], which concluded the toxicated effects of aspartame at lower doses, this study confirmed also that aspartame at doses of 500 mg/day which may be used in food industry as well as medical purposes has a significant cellular toxicity on both liver and kidney tissues.

CONCLUSION

Although aspartame may have a positive effect in obesity as low-calorie, non-weight-bearing dietary alternatives, histological analysis proved that it produces severe cellular toxicity especially in liver and kidney. The toxicological effects performed mechanically via inflammatory and apoptotic pathways. The data recommend that using of aspartame should be taken under medical supervision along with natural antioxidants.

SIGNIFICANCE STATEMENT

This study confirmed that exposure to aspartame is a cellular toxic agent to mice and identified a link between both kidney and liver cell toxicity when animals received aspartame in drinking water at a dose of 500 mg kg^{-1} body for a month. This study contributes to the effective monitoring of other previously reported studies which signify that the consumption of aspartame may lead to severe cellular risk effects with probable incidence of cancer.

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