

Original Research Article

Effect of Aspartame on the Liver of Male Albino Rats: A Histopathological and Immunohistochemical Study

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Abstract

Aspartame is a common artificial sweetener used today. It has a brand name NutraSweet® and Equal®, is produced by union of amino acids, aspartic acid and phenylalanine. It is widely used in many beverages and food while after metabolites in gastrointestinal tract it causes harmful effect to several organs specially the liver, as the liver considered as the largest organ in the abdomen and its function includes working with the pancreas and intestines to process, absorb food, filtering blood from the digestive tract and detoxification of drugs and alcohol. Ki-67 is a nuclear protein antigen which is related to and may be important for proliferation of cells. The aim of the study was to detect the effect of aspartame on the hepatic tissue of male adult rats by noticing any histopathological change and also to observe the localization of Ki-67 protein after giving aspartame orally. Fifty male albino rats used in this study, they were divided into two groups: Group A 10 rats, were received distilled water and regarded as control. Group B 40 rats, administered 40mg/Kg aspartame powder after dissolving it in 2ml distilled water orally daily for six weeks and regarded as treated group. After completion of the experiment the animals were sacrificed. Pieces of liver were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections, which were 4-5 micrometers thick, made then stained with haematoxylin and eosin for histo-pathological examination. Immunohistochemical examination was done by using Ki-67 antibody and the standard streptavidin-biotin immunoperoxidase method. In the treated group, the hepatic plates lost their normal radial arrangement around central veins. The hepatocytes lost their polygonal shape and showed many cytoplasmic vacuoles of variable size; while the localization of Ki-67 was significantly increased in treated group rats. In conclusion receiving aspartame at a dose 40 mg/kg causes histopathological alterations of the hepatic tissues and leads to increase in the proliferation rate.

Key Words: Liver, aspartame, Ki-67.

تأثير الأسبرتام على كبد ذكور الجرذان البيضاء: دراسة أمراضية - نسيجية وكيميائية - نسيجية - مناعية

الخلاصة

الأسبرتام هو محلول اصطناعي موجود تحت الأسماء التجارية NutraSweet® و Equal® وتتكون من اتحاد الأحماض الأمينية وحامض الأسبارتيك والفينيل ألانين. وهي تستخدم على نطاق واسع في العديد من المشروبات والمواد الغذائية، حيث عند اذابة في الجهاز الهضمي يمكن لمكوناته ان تسبب تأثيرات ضارة لعدة اعضاء الجسم خصوصا الكبد، والكبد يعتبر اكبر عضو في البطن وظيفتها العمل مع البنكرياس والأمعاء لمساعدة امتصاص الطعام، وتصفية الدم وإزالة السموم والكحول. أنتيجن Ki-67 هو بروتين نووي له تأثير في زيادة تكاثر الخلايا والغرض من هذا البحث هو تقييم التغيرات نسيجية لكبد الجرذان وتحديد البروتين Ki-67 بعد تناول الأسبرتام، هذه الدراسة اجريت على خمسين من ذكور الجرذان، والجرذان قسمت الى فئتين، فئة السيطرة مكونة من 10 جرذان تناولت ماء المقطر 2مل والفئة الثانية 40 جرذان تناولت الاسبرتام بجرعة 40 مج / كج بعد اذابة في الماء مقطر لفترة ستة اسابيع. اثبتت النتائج ان استخدام الأسبرتام يسبب فقدان صفائح الكبدية ترتيبها شعاعي طبيعي حول الوريد المركزي. ويبدو أن خلايا الكبد تفقد شكلها المصلي وأظهرت العديد من فجوات السيتوبلازمية في خلايا الكبدية مع عدم انتظام في غلاف النواة. واثبتت النتيجة ازدياد ملحوظا على بروتين Ki-67 للمجموعة التي تلقت اسبارتم مقارنة بمجموعة السيطرة. و اثبتت الدراسة ان استخدام الاسبرتام بهذه الجرعة يؤدي الى التغيرات على أنسجة الكبد، لهذا السبب يجب التقليل من تناول الأسبرتام، او استخدامه لفترات قصيرة.

Introduction

Aspartame is a well-known sweetener throughout the world; it is found in many different products of food and is widely used as a tablet for sweetening [1]. The use of an artificial non-carbohydrate sweetener was all the time demanded by many around the world. Populations on diet who need a sweetener with lower calories than sugar, diabetics and others are examples of those consuming this agent. So, there is a rapid development in its consumption over years [2]. This product is 180-200 times sweeter than sugar, doses lower than that of sugar is required for giving the same degree of sweetness so this small doses are giving much less calorie hence it is energy value 4 Kcal/gm. Aspartame is rapidly metabolized in the gut and hydrolyses totally in to the three chief products: (50%) phenylalanine, (40%) aspartic acid and (10%) methanol [3]. Phenylalanine is thought to mediate or exacerbate hepatic encephalopathy, and an impaired liver may not be able to cope with the ammonia genic properties of the amino acid constituents, or adequately metabolize methanol [4]. Methanol can be toxic in high amounts while aspartic acid and Phenylalanine are amino acids which are present in a lot of foods which contain protein but at a high dose can have harmful effects on many organs such as kidneys and liver [5].

The liver forms part of the gastrointestinal system, which is responsible for breaking down, Production, secretion of bile, digestion and absorption, Production of urea, a waste product [6].

Ki-67 is a nuclear protein which is associated with proliferation of cells; it is related to the transcription of rRNA. Inactivation of Ki-67 antigen causes a decrease in synthesis of rRNA [7]. The Ki-67 antigen, during interphase, can be detected in the nucleus of cells, on the other hand during mitosis majority of the protein is relocated to the chromosomes's surface. Ki-67 is observed in all active

phases of cell cycle (G_1 , S, G_2 , and mitosis), but it is not present in resting cells [8, 9].

The aim of the present study is to evaluate the effect of aspartame on the hepatic tissue of rats by noticing any morphological changes and also to detect localization of Ki-67 protein immunohistochemically after administration of aspartame.

Materials and Methods

The present study was performed in the department of Anatomy and Histology at College of Medicine/Hawler Medical University. Fifty adult male albino rats weighing (225-250 grams) were included in this study under standard appropriate laboratory conditions. All the experiment in the present study was done in the period from Feb 2016 to July 2016. Aspartame powder were used for the treated groups. The present study was approved by Ethics Committee of the College of Medicine, Hawler Medical University, Iraq.

The animals were classified into two groups:

Group A: composed of 10 rats control group which received distilled water.

Group B: composed of 40 rats administered 40 mg/Kg aspartame after dissolving it in 2 ml of distilled water orally daily for six weeks.

After finishing of the experiment the rats were sacrificed, parts of the liver were fixed in 10% neutral buffered formalin and embedded in wax. Sections made were 4-5 micrometers thick and then stained by:

1-Haematoxylin and Eosin: for histopathological examination.

2- Immunohistochemistry was performed for localization of Ki-67 using the avidin-biotin-peroxidase complex in which primarily monoclonal antibodies raised against Ki-67 was used. Positive localization of Ki-67 gives nuclear staining of brown color.

The histo-morphological changes were measured for 10 random selected microscopic fields, then the mean of the

measurements were calculated for each case in control and treated groups.

WHO (World Health Organization) classification of 2010 as in Table 1.

Immunohistochemical grading was assigned to each case according to the

Table 1: Grading of Ki-67 according to WHO

Grades	Ki-67 per 10 HPF
Grade 1	< 2
Grade 2	2-20
Grade 3	>20
HPF: high power field	

Statistical analysis were determined using Chi-square (χ^2), and considered statistically significant at the $P < 0.05$

Results

Histopathological examination

The present study showed that the liver of control rats revealed the normal histological structure of hepatic lobule, which formed of central vein and radially arranged hepatocytes (Fig1). The hepatocytes revealed clear cellular boundaries polygonal shape joined to one another and organized in clearly distinguishable plates. Their nuclei were clearly visible. Some cells were binucleated. The endothelial lining of the blood vessels was clearly identified. Portal triad was present between hepatic lobules (Fig-2). While liver of experimental group showed great alterations in the histological

configuration, the liver parenchyma became disorganized; the hepatic tissue plates lost their ordinary radial organization around central vein (Fig3). The liver cells lost their polygonal shape and showed many cytoplasmic vacuoles of different size (Fig 4). The nuclei appeared pyknotic, fragmented and hyperchromatized. binucleated cells were plenty; the blood sinusoids were occluded with blood and congested (Fig 5). Inflammatory cell infiltrate were observed in the parenchyma (Fig 6). These changes were more clear in the intermediate and peripheral zones of liver lobules than that of the central zone. All histo-morphological changes illustrated in the table 2.

Table 2: Morphological changes of the liver tissue in control and treated groups.

Histo-morphological changes	Control group Field/case (mean)	Treated group Field/case (mean)	Pearson χ^2 value	P. value
Normal histological structure	10	0	40.51	0.000
Lost normal radial arrangement of hepatic plates	0	7.35		
Hepatocytes appeared to lose their polygonal shape	1.67	6.79		
Cytoplasmic vacuoles	0	6.12		
Binucleated cells	2.36	5.73		
Blood sinusoids were congested	0	5.92		
Inflammatory cell infiltrate	0	6.47		
Pyknotic nuclei	0	4.88		

Immunohistochemical examination:

The liver tissue of control rats, showed brown staining Ki-67 of some nuclei of hepatocytes (Fig7). Most of nuclei of the liver cells showed grade 1 immune expression of Ki-67. In treated group the number of Ki-67 positive cells was

significantly increased after administration of aspartame for six weeks (Fig 8), and most of nuclei of liver cells showed grade 3.

These results were illustrated and summarized in table (3) and graph (1)

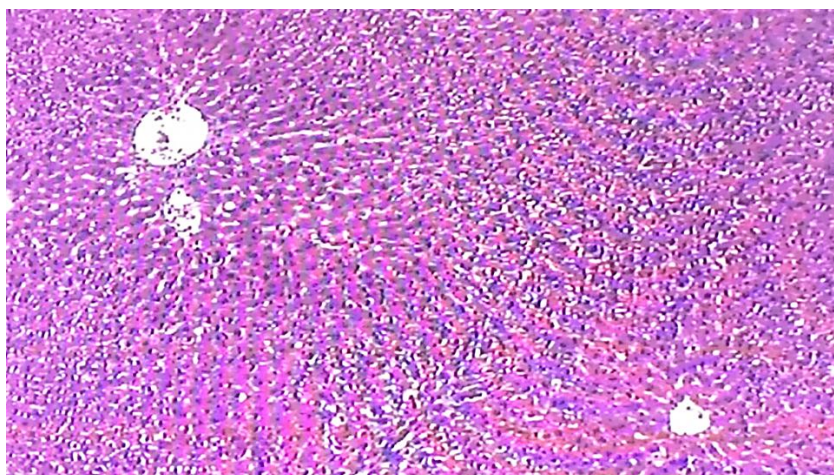


Figure 1: Normal section of rat's liver in the control group (H & E X 100).

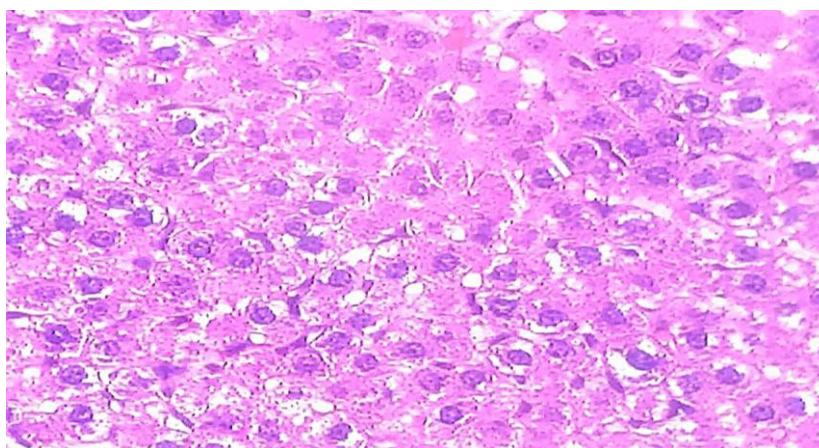


Figure 2: Liver section of control group which shows: clear cell boundaries with central rounded nuclei two or one (H&E X 400)



Figure 3: liver section of experimental rats which shows hepatic parenchyma became disorganized and showed degenerative changes (black arrow), with the loss of normal radial organization of hepatic plate around the central vein. (H&E X 100)

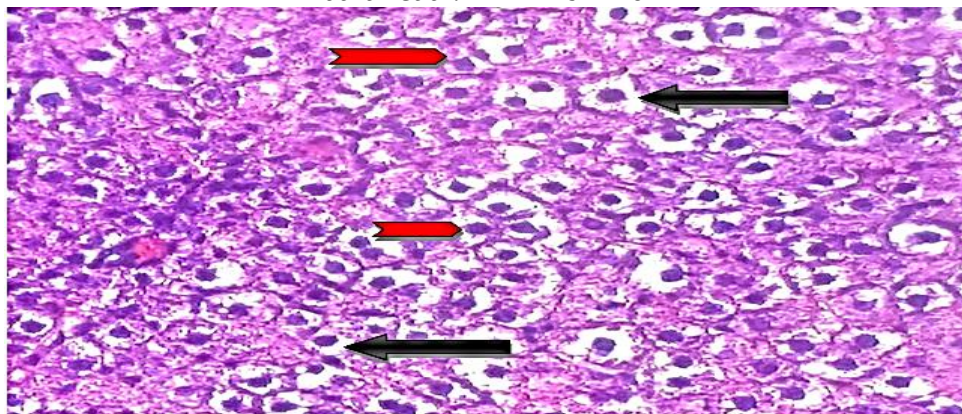


Figure 4: liver section of experimental rats which shows liver cells lost their polygonal shape and many different sized cytoplasmic vacuoles (black arrow). Their nuclei appeared pyknotic and hyperchromatized (red arrow) (H & E X 400)

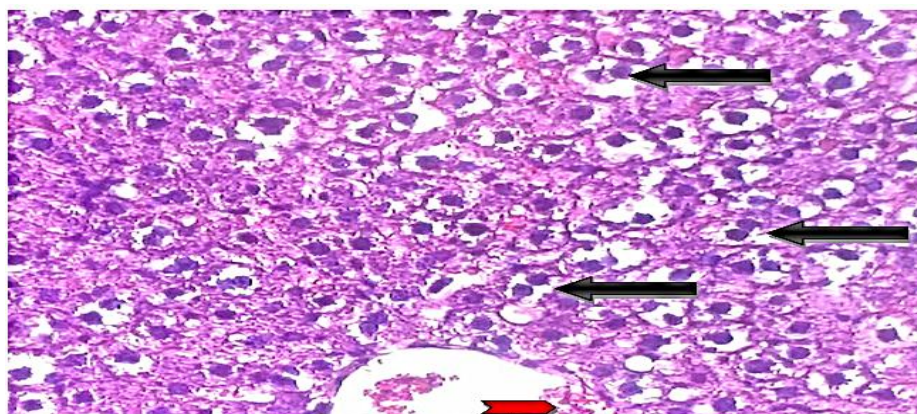


Figure 5: Liver section of experimental rats which shows numerous binucleated hepatocytes (black arrow), the sinusoids are congested and occluded with blood (red arrow) (H & E X 400)

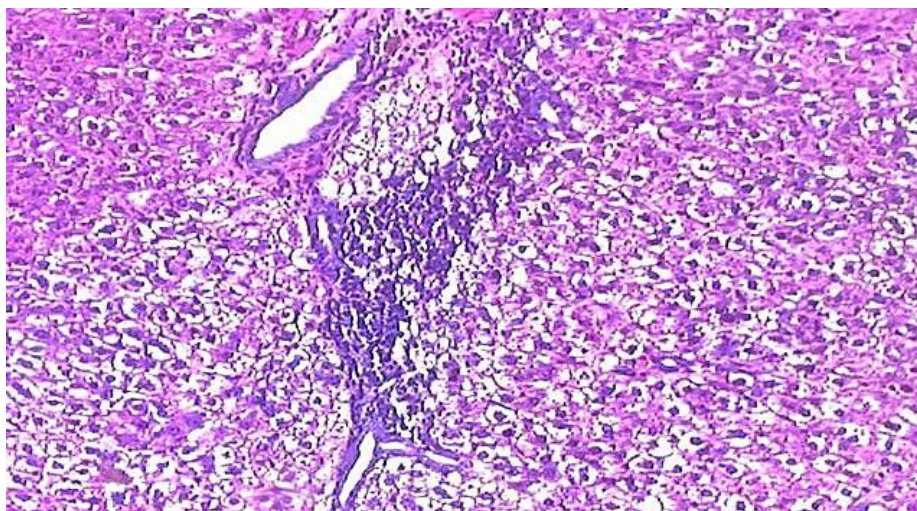


Figure 6: Liver section of experimental rats which shows: portal area congested and infiltrated with many inflammatory cells (H & E x 400)

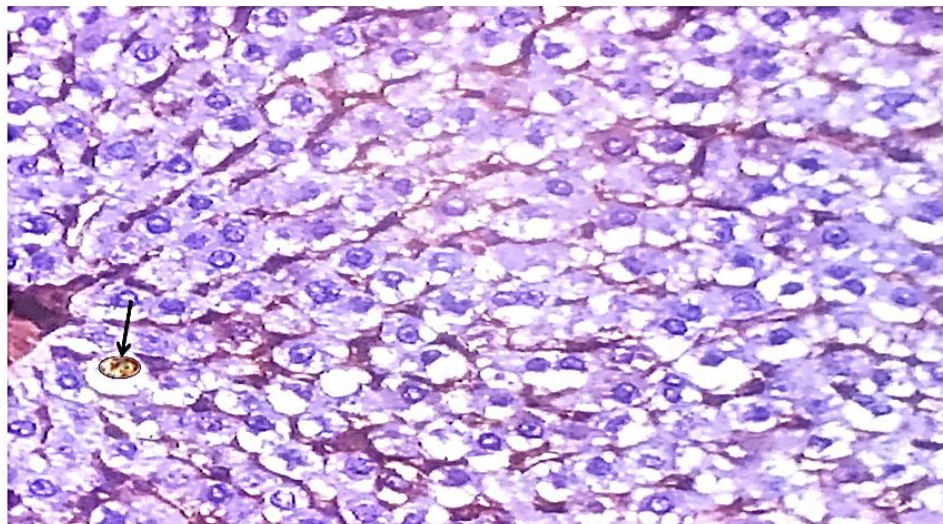


Figure 7: Section from male control liver rat showing a single localization of Ki-67 with brown color (Black arrow). IHC X400

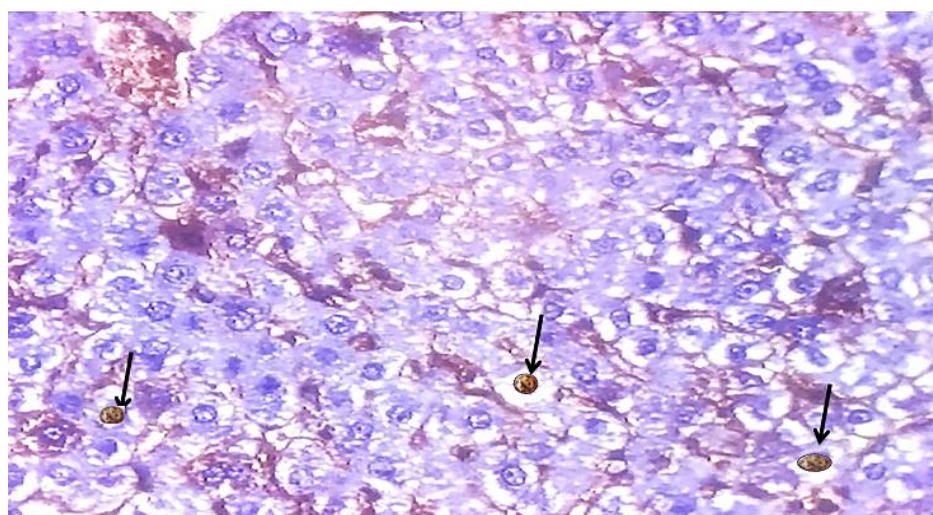
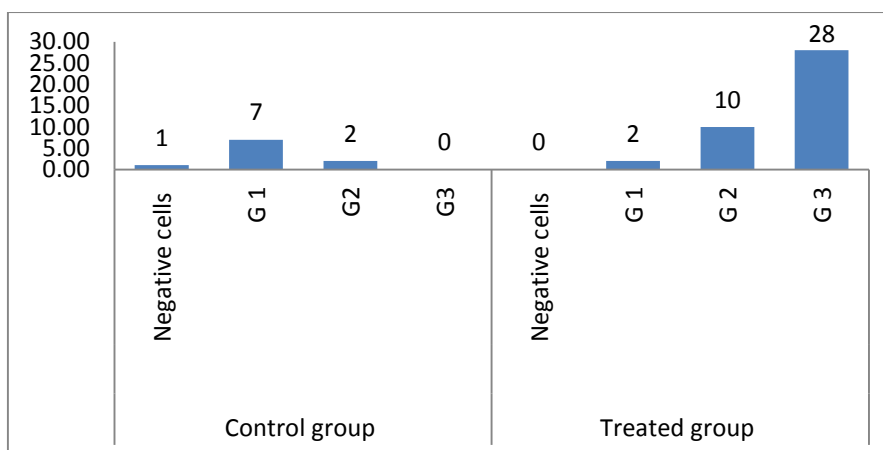


Figure 8: Section from male treated liver rat showing number of positive nuclei localization of Ki-67 with brown color (Black arrow). IHC X400

Table 3: Immunohistochemical expression of Ki-67 antibodies in control and treated groups.

Ki-67	Control group No. 10 cases	Treated group No. 40 cases	Pearson X^2 value	P. value
-ve	1	0	29.861	0.000
Grade 1 (+1)	7	2		
Grade 2 (+2)	2	10		
Grade 3 (+3)	0	28		

Graph1: Grading of immunohistochemical expression of Ki-67 in control and treated rats



Discussion

In this study, the aspartame administration caused great changes in the microscopic structure of the liver tissue, most of the hepatic plates lost their ordinary organization around the central veins, which agrees with Abdallah and Abd Elfatah *et al*. Several mechanisms are involved in the toxic effects of aspartame on the liver. It is shown that after receiving aspartame, it is hydrolyzed in the alimentary tract into three ingredients: methanol, phenylalanine and aspartic acid, the major effects of methanol are found mainly in the kidneys and liver although it has a slow rate of oxidation [13].

Histological examination showed there was vasculations in the cytoplasm of the hepatocytes which is responsible for physical changes in the structure of the plasma membranes and lipids and proteins of membranes of many organelles. This affects the Na⁺/K⁺ pump which lead to the collection of sodium and results in migration of water to the cells. This may be as a result of release of the free radicals which is caused by the production of aspartic acid and methanol after ingestion of aspartame [14].

Methanol may cause increase in lipid peroxidation products on the other hand this may cause damage of the hepatocytes cell membrane [15]. It is reported by some researchers that this vacuoles perhaps represented a cellular defense mechanism against the toxic materials, and this

materials were collected inside the vacuoles so preventing their interference with metabolism of the cells [16].

The binucleated cells and degenerative changes observed in the experimental group of present study, mimics the observation of Osfor and Elias (2003); Stevens and Lowe (2005), Ross and Pawlina (2011) analyzed the pattern of distribution of degeneration. They showed that the liver cells in the peripheral zone were the first to receive toxin, oxygen and nutrients from the sinusoidal blood. These cells are first to be damaged in inflammatory liver disorders while the increase in the number of binuclear hepatocytes in treated group compared to controls which may be attributed to increase mitosis for the repair the damaged hepatocytes.

Liver sections of treated rats showed that the portal area was infiltrated with inflammatory cells and vascular congestion in the portal vein, this is in agreement with the Humphries *et al* who observed paranchymatous hepatitis and accumulation of cellular infiltrate in the liver tissues of adult male rats treated with aspartame. The vascular congestion and dilatation was shown by Barua and Bal after aspartame administration, they attributed this changes to the liberation of Formaldehyde and it is cytotoxicity to the endothelial cells which caused lack of clotting factors and caused bleeding tendency.

Regarding the Ki-67, Ito *et al* indicated that localization of Ki-67 with its levels are critical in evaluating chemical carcinogenesis in liver of rat and it is present during all active phases of cell cycle that makes it a good marker for neoplasia.

The present study shows a clear nuclear staining for Ki-67 in hepatocytes of all rats treated with aspartame. The numbers of Ki-67 positive cells were significantly increased with P value 0.00 of aspartame treated group after six weeks. Ki-67 is useful for proliferative cell activity assessment of liver cells and their expression were higher in treated group. The immune-expression of Ki-67 in the nuclei of the liver cells in treated group could be an indication for raising the proliferation rate which is in an attempt to repair the damaged cells.

Pusztai *et al* said that the accelerated proliferation may indicate an increased mutagenic risk on cells. Ki-67 is involved in cellular cycle could be identified in replicating cells of both malignant and benign lesions

Conclusions

The present study showed that aspartame intake causes histopathological alterations in the liver tissue and increase in proliferation rate of liver cells. So, it is recommended that receiving aspartame should be restricted.

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