Cytotoxic Effect of Aspartame (Diet Sweet) on the Histological and Genetic Structures of Female Albino Rats and Their Offspring

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Abstract: The present study evaluated the effect of aspartame intake on the histological and genetic structures of mother albino rats and their offspring. Sixty adult female albino rats and 180 of their offspring were equally divided into two groups (control and treated), each group divided into three subgroups. Each subgroup consisted of 10 pregnant rats and 30 of their offspring. The experimental design divided into three periods: (1) the gestation period (subgroup one), (2) the gestation period and three weeks after delivery (subgroup two) and (3) animals in the third subgroup treated as subgroup two then left till the end of the ninth week after delivery. Each pregnant rat in the treated subgroups was given a single daily dose of 1 mL aspartame solution (50.4 mg) by gastric gavage throughout the time intervals of experimental design. At the end of each experimental period for control and treated subgroups, the liver of half of both control and treated groups were subjected for histological study while the liver and bone marrow of the other halves were subjected for cytogenetic studies. Body weight of both groups were recorded individually twice weekly in the morning before offering the diet. The results revealed that the rats and their offspring in the subgroups of control animals showed increases in body weight, normal histological sections, low chromosomal aberration and low DNA fragmentation. The treated animals in the three subgroups rats and their offspring revealed decreases in body weight, high histological lesions, increases in the chromosomal aberration and DNA fragmentation compared with control groups. In conclusion, the consumption of aspartame leads to histopathological lesions in the liver and alterations of the genetic system in the liver and bone marrow of mother albino rats and their offspring. These toxicological changes were directly proportional to the duration of its administration and improved after its withdrawal.

Key words: Cytogenetics, histology, chromosome, food additives, aspartame

INTRODUCTION

Food additives are chemicals that are added to food to improve its shelf-life, appearance and flavour (Ishiwata and Simon, 2003). Vencees-Mejia et al. (2006) reported that sweeteners food additives featured reduction in sugar consumption, decrease in caloric intake and maintaining the desirable palatability of foods and soft drinks. Sweeteners are also of primary importance as part of nutritional guidance for diabetes, a disease with increasing incidence in developing as well as developed countries (Gougeon et al., 2004). Aspartame (L-aspartyl L-phenylalanine methyl ester) is a dipeptide artificial sweetener that is widely used (62%) as a non-nutritive sweetener in foods, drinks and pharmaceuticals (Rencuzogullari et al., 2004; Fry, 1999). Following aspartame consumption, its metabolites to phenylalanine, aspartic acid and methanol are increased in the blood (Ranney et al., 1976; Stegink, 1987).

Previous studies on aspartame had been carried out to understand its effect as cancer factor and neurotoxicity mechanism (Christian et al., 2004; Tsakiris et al., 2005; Soffritti et al., 2006; Bergstrom et al., 2007; Gallus et al., 2007). Despite numerous toxicological studies of aspartame (Simuntzi et al., 2007), its effects on hepatic tissue have received little attention. So, there is a need to substantiate whether long term oral consumption of aspartame induces oxidative stress and structural changes in hepatic tissue.

The purpose of this study was that: (1) Detection of the effect of aspartame on the histological structure of the liver of mother albino rats and their offspring compared to
the control and (2) Determination of the cytogenetic effects of aspartame on the bone marrow and liver cells of mother albino rats and their offspring compared to the control.

MATERIALS AND METHODS

Drug: Aspartame was purchased from Amriya Company (Egypt). Aspartame was available in the form of tablets, each contains 20 mg.

Dose calculation, drug preparation and administration: The human maximum ADI of aspartame was estimated as 40 mg kg\(^{-1}\) b.wt. (FAO/WHO, 1980). In this study, the equivalent dose for an adult rat weighting about 200 g was calculated by using the formula of Paget and Barnes (1964) to be 50.4 mg/rat. Milling of the tablets to the form of powder was done. For each pregnant rat a daily dose of 50.4 mg was weighted, dissolved in 1 mL of distilled water and administrated to the pregnant rat orally through gavage tube.

Experimental animals and design: Eighty adult albino rats (60 females and 20 males, average of 180±20 g) were used in this study. Throughout the experiment, all animals were observed and maintained on balanced diet and water (Standard diet pellets-El-Nasr-Company, Abou-Zaabal-Egypt).

The adult females were isolated from the adult males for two weeks before the beginning of the experiment. Each male was kept overnight with three females in a separate cage. Next morning, all females showing vaginal plugs were considered to be in the first day of pregnancy (Barcollona et al., 1977). The pregnant rats were kept in separate cages for continuation of the experiment and the offspring were kept with their mothers for feeding.

The 60 pregnant rats and 180 of their offspring were equally divided into two groups (control and treated), each group divided into three subgroups. Each subgroup consisted of 10 pregnant rats and 30 of their offspring. The experimental design divided into three periods: (1) the gestation period (subgroup one), (2) the gestation period and three weeks after delivery (subgroup two) and (3) animals in the third subgroup treated as subgroup two then left till the end of the ninth week after delivery. Each pregnant rat in control group was given a single daily dose of 1 mL distilled water by gastric gavage during the time intervals of experimental design. Each pregnant rat in the treated subgroups was given a single daily dose of 1 mL aspartame solution (50.4 mg) by gastric gavage throughout the time intervals of experimental design.

At the end of each treatment, the liver of half of both control and treated groups were subjected for histological study, while the liver and bone marrow of the other halves were subjected for cytogenetic study. Body weight of both control and treated groups were recorded individually twice weekly in the morning before offering the diet.

Histological study

Specimen collection: The mothers and their offspring which were used for the histological study were sacrificed under mild diethyl ether anesthesia. For each rat, the abdominal cavity was exposed by midline incision and the liver was dissected and collected. The specimens were immediately fixed by immersion in 10% formal saline solution. Haematoxylin and eosin stain (Kieran, 2001) were used to study the general histological structure of the liver. In addition, masson's trichrome stain (Kieran, 2001) was used to demonstrate the collagen fibers in the liver. Periodic Acid Schiff reaction (PAS) technique (Bancroft and Steven, 1996) for the study of mucopolysaccharides and polysaccharides was carried out.

Cytogenetic study: This study was done using chromosomal aberrations study and quantitation of DNA fragmentation. Chromosomal aberration study was done using somatic cell analysis on the bone marrow of pregnant rat and liver of offspring. The bone marrow and liver cells were processed using the method described by Rabello-Gay and Ahmed (1980).

Metaphase and chromosomal detection: Scanning slides for mitotic spread metaphases were conveniently accomplished with a 25x magnification objective and analyzed with a 100x oil objective. One hundred well spread metaphases per rat were examined for numerical as well as structural aberrations.

Quantitation of DNA fragmentation: This test was carried out on the liver cells of all experimental chosen for cytogenetic study. Quantitation of DNA fragmentation was determined via the colorimetric diphenylamine assay as described by Gibb et al. (1997).

Statistical analysis: All data were tabulated and statistically analyzed using the arithmetic mean, standard deviation, Student t-test (unpaired) and Analysis of Variance (ANOVA), Posthoc multiple comparisons tests (Pipkin and Livingstone, 1984).
RESULTS

Body weight study:
In mother rats: At the first day of pregnancy, the mean values of the initial body weight for mothers were ranged from 185 to 189 g. At the end of the experiments, there was highly significant decrease of the final body weight of treated rats compared with control group. The percentage of reduction of the body weight in the treated three subgroups compared with the three control subgroups were 8.1, 10.28, 4.71%, respectively (Table 1).

In offspring rats: At the end of experiments, the mean values of body weight for the offspring revealed highly significant decrease of the body weight of the treated subgroups as compared to the control subgroups respectively. The percentage of reduction of the body weight in the three treated subgroups in relation to the three control subgroup respectively were 8.4, 10.8 and 4.6%, respectively (Table 2).

Histological study
In mother rats: Examination of serial transverse sections of the liver of control mothers showed no detectable differences in the histological structure of their liver. The liver was formed of hepatocytes arranged in branching, interconnecting cords of one cell thick. The hepatic cords were radiating from the central vein and interposed with hepatic sinusoids. The cytoplasm of the hepatocytes was acidophilic with small scattered basophilic bodies (Fig. 1a, b). Blood sinusoids were situated between the cords of the hepatocytes and were lined by flat endothelial cells and larger, darker Von Kupffer cells (Fig. 1a). Masson’s trichrome stain showed the normal distribution of the collagen fibers in the portal area and in between hepatocytes (Fig. 1c). Periodic acid Schiff reaction showed the normal distribution of PAS positive material in the hepatocytes (Fig. 1d).

Examination of serial transverse sections of the liver of mothers treated throughout the period of gestation (mothers of the 1st treated subgroup) showed that the hepatic parenchyma became disorganized where most of the hepatic cords lost its normal radial arrangement around the central vein (Fig. 1e). Most of the blood sinusoids became narrow and some of them appeared congested compared with the control mothers group. Most of the hepatocytes showed moderate degenerative changes which were more prominent in the peripheral and intermediate zones of the hepatic lobule than that of the centrolobular zone. Most of the hepatocytes lost their characteristic polygonal shape and became rounded, oval or ballooned. Also, the cytoplasm of the majority of the hepatocytes showed numerous variable sized vacuoles that in some cells became large enough to replace most of the cytoplasm (Fig. 1e). The nuclei of some hepatocytes assumed one of four patterns of degeneration; hyperchromatism, pyknosis, fragmentation and in the fourth pattern, the nucleus in a dead cell completely disappeared (Fig. 1f). Masson’s trichrome stain showed moderate increase in the collagen fibers deposition around the portal tract and in between the hepatocytes (Fig. 1g) compared with the control mothers group. Periodic Acid Schiff reaction showed moderate reduction of the distribution of the PAS positive material in the hepatocytes (Fig. 1h) compared with the control mothers group.

Examination of serial transverse sections of the liver of mothers treated throughout the period of gestation and three weeks after delivery showed that the hepatic parenchyma lost its normal architecture. Most of the hepatic cords became disorganized and lost its radial arrangement around the central vein. The blood sinusoids were narrow and slightly congested (Fig. 2a) as compared with the control mothers group (Fig. 1a, b). Disruption of the hepatic cords and blood sinusoids by a circumcribed cellular mass adjacent to the portal tract was detected. Most of the hepatocytes showed severe degenerative

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Treated</th>
<th>Reduction from control (%)</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>6.6±0.316</td>
<td>6.64±0.22</td>
<td>8.4</td>
<td>5.587</td>
</tr>
<tr>
<td>2nd</td>
<td>27.7±1.090</td>
<td>24.7±1.210</td>
<td>10.8</td>
<td>6.085</td>
</tr>
<tr>
<td>3rd</td>
<td>95.28±0.44</td>
<td>90.9±0.894</td>
<td>4.6</td>
<td>9.837</td>
</tr>
</tbody>
</table>

Values are Mean±SD. **Means highly significant difference at p<0.001
Fig. 1(a-h): (a-d) Photomicrograph of a transverse section of the liver of control mother and (e-h) Mother treated throughout the period of gestation. CV: Central vein, L: Lymphatic duct, H: Hepatocytes, HC: Hyper chromatic nucleus, S: Sinusoids, P: Pyknotic nucleus, K: Kupfer cell, V: Vacuolated cytoplasm, E: Endothelial cell, M: Mononuclear cell infiltration, BD: Bile duct.
Fig. 2(a-j): (a-f) Photomicrograph of a transverse section of the liver of mother treated throughout the period of gestation and three weeks after delivery and (g-j) Mother of the third treated group at the end of the 9th week after delivery (g-j). CV: Central vein, L: Lymphocyte, Pt: Portal tract, HC: Hyper chromatic nucleus, E: Eosinophil, M: Macrophage, P: Plasma cell, Mu: Multinuclear giant cell, Black arrow, †: Pyknotic nucleus, Red arrow, ‡: Fragmented nuclei, Yellow arrow, ††: Lost nuclei, M: Mononuclear cell infiltration, F: Fibroblast, G: Cellular mass
changes which were more prominent in the peripheral and intermediate zones of the hepatic lobule than that of the central lobular zone (Fig. 2b). The cytoplasm of the majority of the hepatocytes looked empty and contained only scattered wisps of cytoplasmic remnants (Fig. 2a, b). The nuclei of the majority of the hepatocytes showed variable degrees of degeneration ranging from hyperchromatism, pyknosis, fragmentation to complete disappearance (Fig. 2b). The inflammatory infiltrate spilled over into the adjacent hepatic parenchyma forming a circumscribed cellular mass consisted of mononuclear and multinuclear cells. The mononuclear cells were lymphocytes, macrophages, eosinophils, fibroblasts and plasma cells while the multinuclear cells were multinuclear giant cells (Fig. 2c, d). As regard the multinuclear cells, the multinuclear giant cell was large oval cell with acidophilic cytoplasm and multiple nuclei. Masson's trichrome stain showed massive increase in the collagen fibers deposition around the portal tract and in between the hepatocytes (Fig. 2e). Periodic Acid Schiff reaction showed marked reduction in the distribution of PAS positive material in the hepatocytes (Fig. 2f) as compared with the control mothers group (Fig. 1d).

Examination of serial transverse sections of the liver of mothers of the third treated group at the 9th weeks after delivery showed that the hepatic parenchyma mostly restored its normal architecture. Most of the hepatic cords restored its radial arrangement around the central vein (Fig. 2g). The blood sinuoids were narrow (Fig. 2g) as compared with the control mothers group (Fig. 1a). Some hepatocytes showed variable sized vacuoles in their cytoplasm. Few nuclei showed variable degrees of degeneration ranging from hyperchromatism, pyknosis, to fragmentation (Fig. 2h). Masson's trichrome stain showed moderate increase in the deposition of collagen fibers in the portal and periporal areas (Fig. 2i) as compared with the control mothers group (Fig. 1c). Periodic Acid Schiff reaction showed restoration of most of the distribution of PAS positive material in the hepatocytes (Fig. 2j) as compared with the control mothers group (Fig. 1d).

In offspring rats: Examination of serial transverse sections of the liver of control offspring at birth showed that the liver parenchyma consisted of hepatocytes aggregated as irregular groups or arranged as intercommunicating cords of one or two cell thick plates, rarely radiating from the central vein and separated by primitive blood sinusoids. The latter were varied in size and were lined with scanty endothelial cells which were difficult to be detected as they were masked by islets of haemopoietic cells which were dispersed throughout the liver parenchyma. The portal area consisted of a branch of portal vein and a branch of bile duct (Fig. 3a). Masson's trichrome stain and Periodic acid Schiff reaction (Fig. 3b) showed the normal distribution of the collagen fibers and PAS positive material respectively (Fig. 3c).

Examination of serial transverse sections of the liver of three weeks old control offspring showed that the hepatic parenchyma became more developed than in offspring of the first control group. The parenchyma was formed of cords of hepatocytes (one or two cells thick) regularly radiating from the central vein and separated by blood sinuoids. The latter were lined with flat endothelial cells and Von Kupffer cells which could be easily detected (Fig. 3d). The haemopoietic islets could not be detected at this age. The portal area (Fig. 3e) consisted of a branch of portal vein and a branch of bile duct. Masson's trichrome stain and Periodic acid Schiff reaction (Fig. 3f) showed the normal distribution of the collagen fibers and PAS positive material, respectively (Fig. 3g).

Examination of serial transverse sections of the liver of offspring of the 1st treated group showed that some groups of hepatocytes appeared nearly similar to their control. Other groups showed variable signs of moderate degenerations. Some of the degenerated hepatocytes had ill-defined or even ruptured cell boundaries and the cytoplasm of the majority of them contained variable sized vacuoles that in some cells became large enough to replace most of the cytoplasm (Fig. 4a). The nuclei of the degenerated hepatocytes either appeared rounded and vesicular or showed variable degrees of degeneration and necrosis ranging from pyknosis, fainting to complete disappearance (Fig. 4a, b). The blood sinuoids were slightly congested as compared to the control group while the haemopoietic islets (Fig. 4a, b) appeared nearly similar to their control (Fig. 3a, b). Marked degenerative changes affecting most of the hepatocytes could also be detected in the periporal area. Masson's trichrome stain showed moderate to marked increase in the collagen fibers deposition around the portal tract and in between the hepatocytes (Fig. 4c) as compared to their control (Fig. 3b). Periodic acid Schiff reaction showed mild reduction of the PAS positive material in the hepatocytes (Fig. 4d) as compared to their control (Fig. 3c).

Examination of serial transverse sections of the liver of offspring of the 2nd treated group showed that the hepatic cords became disorganized and lost its radial arrangement around the central vein. Some blood sinuoids appeared congested while most of them could hardly be detected (Fig. 4e). Most of the hepatocytes showed variable signs of marked degeneration and necrosis. The cell boundaries of some of the degenerated hepatocytes became ill-defined or even ruptured. Most of them lost their characteristic polygonal shape and became
Fig. 3(a-g): (a-e) Photomicrograph of a transverse section of the liver control offspring and (d-g) Offspring of the 2nd control group. CV: Central Vein, H: Hepatocytes, PV: Portal Vein, BD: Bile Duct, S: Sinusoids, K: Kupfer cell, E: Endothelial cell.
Fig. 4(a-h): (a-d) Photomicrograph of a transverse section of the liver of offspring 1st treated group and (e-h) 2nd treated group. CV: Central Vein, M: Mononuclear cell infiltration, V: Vacuolated cytoplasm S: Sinusoids, HI: Haemopoietic islets, PV: Portal Vein, r: ruptured cell boundaries, HC: Hyper chromatic nucleus, Black arrow, l: Pyknotic nucleus, P: Pale, Yellow arrow, l: Lost nuclei, BD: Bile Duct, K: Kupfer cell.
oval, rounded or balloononed. Their cytoplasm either appeared pale or showed numerous variable sized vacuoles that in some cells became large enough to replace most of the cytoplasm. The nuclei of some hepatocytes appeared rounded and vesicular while most of the nuclei showed variable degrees of degeneration and necrosis ranging from hyperchromatism, pyknosis, fainting to complete disappearance (Fig. 4c, f). Congestion of the portal vein, mononuclear cells infiltration of the portal tract and variable patterns of degeneration in the hepatocytes surrounding the portal area which were more prominent than that observed in the pericentral area could also be detected. Masson's trichrome stain showed marked increase in the collagen fibers deposition around the portal tract and in between the hepatocytes (Fig. 4g) as compared with their control. Periodic acid Schiff reaction showed marked reduction in distribution of the PAS positive material in the hepatocytes (Fig. 4h) as compared to their control.

Examination of serial transverse sections of the liver of offspring of the 3rd treated group at 9th week after delivery showed nearly similar results that were observed in their mothers (Fig. 1, 2).

**Cytogenetic study**

**In mother rats:** The results of the chromosomal aberrations in the bone marrow cells of control mother albino rats revealed that there were no significant differences between all control groups.

Administration of aspartame to mother albino rats treated throughout the period of gestation, throughout the period of gestation and three weeks after delivery and the third treated group at the 9th weeks after delivery showed increases in the frequency of structural and numerical chromosomal aberrations in their bone marrow cells compared to the control mothers group. Gaps, deletions, breaks, rings, end to end, hyperdiploidy and hypodiploidy were the detected chromosomal aberrations. It revealed highly significant increase of total chromosomal aberrations 1st, 2nd and 3rd treated groups (6.00±1.41, 6.75±0.50 and 4.50±0.57, respectively) as compared to the control mothers group (2.00±0.00). It also showed significant decrease of total chromosomal aberrations in the 3rd treated group (4.50±0.57) as compared to 1st and 2nd treated groups (6.00±1.41 and 6.75±0.50, respectively). Other relations showed insignificant differences (Table 3).

**In offspring rats:** Administration of aspartame to mother albino rats of the 1st treated group significantly increased some of the structural and numerical chromosomal aberrations in the liver cells of their offspring when it is compared to offspring of the control group (Table 4). Gaps, deletions, breaks, hyperdiploidy and hypodiploidy were the detected chromosomal aberrations.

The result of Table 5 illustrated that the 2nd and 3rd of offspring treated subgroups showed increases in the frequency of total structural chromosomal aberrations (3.60±0.62 and 2.33±0.50, respectively) and hypodiploidy (3.80±1.30 and 3.00±1.11, respectively) compared to the control group (1.87±0.35 and 2.62±0.74, respectively).

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**Table 3: Different structural and numerical chromosomal aberrations induced in the bone marrow cells of mother albino rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of examined cells</th>
<th>Structural chromosomal aberrations</th>
<th>Numerical chromosomal aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gaps</td>
<td>Deletions</td>
</tr>
<tr>
<td>Control mother</td>
<td>500</td>
<td>1.40±0.54</td>
<td>0.60±0.54</td>
</tr>
<tr>
<td>1st treated</td>
<td>500</td>
<td>2.00±0.81</td>
<td>2.00±0.81</td>
</tr>
<tr>
<td>2nd treated</td>
<td>500</td>
<td>1.54±1.00</td>
<td>2.75±0.50</td>
</tr>
<tr>
<td>3rd treated</td>
<td>500</td>
<td>1.25±0.50</td>
<td>1.75±0.50</td>
</tr>
<tr>
<td>ANOVA</td>
<td>F</td>
<td>0.80±1.12</td>
<td>0.52±1.25</td>
</tr>
</tbody>
</table>

ANOVA (one way statistical analysis) comparing between the control mothers and 1st, 2nd and 3rd treated groups regarding different structural and numerical chromosomal aberrations. P<0.05 means insignificant difference; P<0.025 means significant difference; P<0.01** means highly significant difference. Values are Mean±SD

**Table 4: Different structural and numerical chromosomal aberrations induced in the liver cells of offspring albino rats at birth**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of examined cells</th>
<th>Structural chromosomal aberrations</th>
<th>Numerical chromosomal aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gaps</td>
<td>Deletions</td>
</tr>
<tr>
<td>1st control group</td>
<td>1500</td>
<td>1.55±0.52</td>
<td>0.44±0.52</td>
</tr>
<tr>
<td>1st treated group</td>
<td>1500</td>
<td>1.71±0.40</td>
<td>1.09±0.70</td>
</tr>
<tr>
<td>T-test</td>
<td>T</td>
<td>-1.262</td>
<td>-2.35</td>
</tr>
<tr>
<td>p-value</td>
<td>0.23</td>
<td>0.03**</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Student "T" test comparing between 1st control and 1st treated groups regarding different structural and numerical chromosomal aberrations. P<0.05 means insignificant difference; P<0.025 means significant difference; P<0.01** means highly significant difference. Values are Mean±SD
Table 5: Different structural and numerical chromosomal aberrations induced in the bone marrow cells of 3 and 9 weeks old offspring

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of examined cells</th>
<th>Structural chromosomal aberrations</th>
<th>Numerical chromosomal aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gap</td>
<td>Deletions</td>
</tr>
<tr>
<td>Controls</td>
<td>1500</td>
<td>1.75±0.46</td>
<td>0.12±0.35</td>
</tr>
<tr>
<td>2nd treated</td>
<td>1500</td>
<td>1.91±0.51</td>
<td>1.04±0.71</td>
</tr>
<tr>
<td>3rd treated</td>
<td>1500</td>
<td>1.8±0.52</td>
<td>0.44±0.52</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.286</td>
<td>0.020**</td>
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</table>

ANOVA (one way statistical analysis) comparing between the controls, 2nd and 3rd groups, regarding different structural and numerical chromosomal aberrations; p>0.05 means insignificant difference; p<0.05* means significant difference, p<0.01** means highly significant difference, Values are Mean±SD

Table 6: Mean percentage of DNA fragmentation induced in the liver cells of mother albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA fragmentation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mother</td>
<td>3.38±0.65</td>
</tr>
<tr>
<td>1st treated</td>
<td>6.78±0.51</td>
</tr>
<tr>
<td>2nd treated</td>
<td>7.48±1.13</td>
</tr>
<tr>
<td>3rd treated</td>
<td>4.50±0.83</td>
</tr>
<tr>
<td>ANOVA</td>
<td>37.10</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

ANOVA (one way statistical analysis) comparing between control mother, 1st, 2nd and 3rd treated groups regarding DNA fragmentation percentage, p<0.01** means highly significant difference, Values are Mean±SD

Table 7: Mean percentage of DNA fragmentation induced in the liver cells of offspring at birth

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st control</td>
<td>3.76±1.00</td>
</tr>
<tr>
<td>1st treated</td>
<td>5.23±0.84</td>
</tr>
<tr>
<td>t-test</td>
<td>1.833</td>
</tr>
<tr>
<td>p-value</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

Student "t" test comparing between 1st control and 1st treated groups regarding DNA fragmentation percentage, p<0.05* means significant difference, Values are Mean±SD

DNA fragmentation assay

In mother rats: The mean percentage of DNA fragmentation induced in the liver cells of the control mothers (3.38±0.65) and the 1st, 2nd and 3rd treated subgroups (6.78±0.51, 7.48±1.13 and 4.50±0.83, respectively) revealed highly significant differences between treated subgroups and control (Table 6). On the other hand it showed highly significant decrease in 3rd treated subgroup (4.9±0.83) compared to 1st and 2nd treated subgroups (6.78±0.51 and 7.48±1.13, respectively) (Table 6).

In offspring rats: The mean percentage of DNA fragmentation induced in the liver cell of offspring of the control and 1st treated subgroups revealed significant increase of the percentage mean of DNA fragmentation in 1st treated subgroup (5.23±0.84) compared to control group (3.76±1.00) (Table 7).

The percentage mean of DNA fragmentation in the control (3.39±0.94) and 2nd and 3rd (5.8±0.84 and 4.43±0.73, respectively) treated subgroups revealed highly significant differences between these studied groups (Table 8).

Table 8: Mean percentage of DNA fragmentation induced in the liver cells of 3 weeks and 9 weeks old offspring

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.39±0.94</td>
</tr>
<tr>
<td>2nd treated</td>
<td>5.80±0.84</td>
</tr>
<tr>
<td>3rd treated</td>
<td>4.43±0.73</td>
</tr>
<tr>
<td>ANOVA</td>
<td>13.751</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001**</td>
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</table>

ANOVA (one way statistical analysis) comparing between the controls and 2nd and 3rd treated groups regarding DNA fragmentation percentage, p<0.01** means highly significant difference, Values are Mean±SD

DISCUSSION

Aspartame is the most used artificial sweetener in the world. Hundreds of millions of people consume aspartame worldwide; among the major users of aspartame are children and women of childbearing age (Anton et al., 2010; Soffritti et al., 2010). Despite numerous toxicological studies of aspartame, its effects on hepatic tissue have received little attention (Abhulash et al., 2011). Also, consequences of its intake for pregnant women have been minimally addressed (Haldorson et al., 2010). Thus, in view of increasing popularity of aspartame, their various clinical effects with particular regard to their safety require further investigations. The present study was carried out to study the effect of aspartame on the histological structure of the liver and its cytogenetic effect on the liver and bone marrow of albino rat mothers and their offspring. In the current study, administration of aspartame to albino rat mothers and their offspring induced highly significant reduction of their body weight compared to control groups. This result is supported by Astrup et al. (2002) and De La Hunity et al. (2006) who reported that the body weight and the fat mass decreased in overweight subjects supplemented with aspartame for ten weeks.

Also, Portela et al. (2007) observed that, in the 20th day of gestation, there was a highly significant reduction in the body weight of adult female rats treated with aspartame (14 mg kg⁻¹ by gavage) on the 9th, 10th, 11th days of gestation in relation to the control group. In addition, the authors observed significant reduction of the body weight mean of their fetuses in relation to the control group fetal weight.
Many authors described the mechanisms by which aspartame could induce reduction in the body weight. Rogers et al. (1990) mentioned that aspartame induced satiety in human beings and thereby leads to weight loss. Hall et al. (2003) suggested that the satiating effect of aspartame might be due to a post-absorptive effect of rising circulating levels of phenylalanine. The phenylalanine constituent of aspartame had two effects; the first was suppression of food intake in humans and animals (Muurahainen et al., 1988). The second was increase in cholecystokinin secretion which delays the gastric emptying (Ballinger and Clark, 1994). On the other hand, it was noticed that the decrease in the body weight in rats supplemented with aspartame for a period of fourteen weeks started at weaning was associated with diminution of Neuropeptide Y (NPY) in its principal hypothalamic site of synthesis (Beck et al., 2002). NPY promotes weight gain and fat deposition as it both inhibits lipolysis and stimulates denovo lipogenesis (Billington et al., 1991; Zarjeski et al., 1993). So that, the beneficial effects of aspartame on the body weight could be related to the decreased effects of NPY on the lipid metabolism but the reasons for the NPY decrease (physiological or consequence of adverse effects of aspartame) were not obvious (Beck et al., 2002).

In the current study examination of serial transverse sections of the liver of albino rat mothers of the 1st and 2nd treated subgroups showed that the hepatic parenchyma lost its normal architecture where most of the hepatic cords lost its normal radial arrangement around the central vein. The hepatocytes showed moderate and severe degenerative changes in the 1st and 2nd treated subgroups, respectively. These degenerative changes were more prominent in the peripheral and intermediate zones of the hepatic lobule than those of the centrlobular zone.

This pattern of distribution of degeneration was explained by Stevens and Lowe (2005) and Ross and Pawlina (2011), who stated that the hepatocytes in the periportal zone were the first to receive oxygen, nutrient and toxins from the sinusoidal blood. These cells are also the first to be damaged in inflammatory liver disorders that primarily involve the portal tract.

In the present study, mothers of the 1st and 2nd treated subgroups showed that the cell boundaries of most of the hepatocytes became ill defined and in the 2nd treated subgroup they showed rupture. Most of the hepatocytes lost their characteristic polygonal shape and became oval, rounded or balloononed. The cytoplasm of the majority of the hepatocytes contained variable sized vacuoles in 1st treated subgroup while in the 2nd treated subgroup it looked empty and contained only scattered wisps of cytoplasmic remnants.

These results are in line with Osfor and Elias (2003), who stated that examination of liver sections of adult male rats treated with aspartame for six and twelve weeks revealed cloudy swelling of the hepatocytes compared to the control group. The ill defined and ruptured cell boundaries observed following aspartame administration were explained by Iman (2011), who observed significant elevation in lipid peroxidation (LPO) level in the liver tissue of adult male rats after four and six weeks of oral treatment with aspartame. The author mentioned that LPO is an autocatalytic mechanism leading to oxidative destruction of cellular membranes. The latter is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane (Bergendi et al., 1999). These latter compounds then decompose to form a wide variety of products in particular malonaldehyde (MDA) (Zeyuan et al., 1998). The increase in MDA level was an index of indicated liver cell membrane damage after aspartame administration (Iman, 2011).

In the present study, the hepatic parenchyma of the offspring of the 1st and 2nd treated subgroups showed moderate and marked degeneration, respectively. These were clearly detected as hepatic parenchyma lost its normal architecture and in the 2nd treated subgroup the hepatic cords lost its normal radial arrangement around the central vein. These findings are in agreement with Portela et al. (2007), who found that the oestrogastral administration of aspartame to adult female rats on the ninth, tenth and eleventh days of gestation induced fetal hepatic toxicity as evidenced by the significant reduction in the karyometric parameters of fetal hepatocyte nuclei (major, minor, mean diameters; volume, area, perimeter; volume to area ratio and index of contour of hepatocyte nuclei) in relation to the control group. Also, our results are in line with Martin and Azoubel (2007), who showed a statistically significantly increased cell volume and decreased numerical cell density in the fetal kidneys of rats whose mothers treated with aspartame diluted in distal water compared to the control. The authors concluded that the administration of aspartame during pregnancy delayed fetal growth as expressed by cell damage during this period.

In the present study, mothers of the 2nd treated subgroup showed that the inflammatory infiltrate of the portal tract spilled over into the adjacent hepatic parenchyma forming circumscribed cellular mass which became consisted of mononuclear and multinuclear cells. Similar result was observed by Hamoudah (1990), who showed parenchymatous hepatitis and aggregation of cellular infiltrate in the liver tissues of adult male rats treated with aspartame. The authors described the aggregations of cellular infiltrate to be granulomas.
McCance and Grey (2008) mentioned that if the inflammatory cells fail to cooperate and protect the host from tissue damage, the body attempts to wall off and isolate the affected area, thus forming granuloma.

The current study showed increased collagen fiber deposition around the portal tract and in between the hepatocytes in mothers and offspring of the 1st and 2nd treated groups. These findings were most probably due to the effect of the metabolites of aspartame on the cell proteins. This suggestion is in agreement with a study in which it was observed a significant elevation in LPO level in the liver tissue of adult male rats after four and six weeks of treatment with aspartame (Iman, 2011). LPO caused oxidative damage to proteins and nucleic acids and the end results of these reactions increased the collagen and ground substance formation (Bacon and Britton 1989).

The present study showed reduction of the PAS positive material in the liver tissues of mothers and offspring of the 1st and 2nd treated subgroups. This finding was in line with Hamoudah (1990) and Sadek and Aby El-Maksoud (1997), who stated that administration of aspartame to the adult male rats induced reduction of the PAS positive material of the hepatocytes. Hamoudah (1990) mentioned that the glycogenolytic effect of the additive may be due to its direct action on the cell stimulating glycogenolysis or due to its effect on the other cytoplasmic membranes organelles and the associated enzymes necessary for glycogen synthesis.

Chromosomal assay has been used as a biomarker predictor to evaluate health risk of developing neoplasm (Mateca et al., 2006). Cytogenetic analysis of somatic cells of mother albino rats and their offspring was used to assess the genetic risk of aspartame. The analysis included assessment of chromosomal aberrations and DNA fragmentation. The current work showed that administration of aspartame to mothers of the 1st and 2nd treated subgroups increased the frequency of structural chromosomal aberrations in their bone marrow cells. This could be easily observed as the bone marrow cells of mothers of the 1st treated subgroup showed highly significant increase of deletions and total structural chromosomal aberrations compared to control mothers group. Also, it showed significant increase of peridiploid compared to control mothers group. On the other hand, the bone marrow cells of mothers of the 2nd treated subgroup showed highly significant increase of deletions, breaks, total structural chromosomal aberrations and peridiploid compared to the control mothers group. Also, liver cells of the offspring of the 1st treated subgroup showed highly significant increase of the total structural chromosomal aberrations and significant increase of both deletions and peridiploidy compared to the offspring of the control group. On the other hand, the bone marrow cells of the offspring of the 2nd treated subgroup showed highly significant increase of deletions and total structural chromosomal aberrations. Also, it showed significant increase of breaks and peridiploidy compared to the offspring of the control group.

This result is in line with some previous studies done on aspartame. Al-Shaibaani (2010) observed that aspartame induced a significant increase of chromosome aberration frequencies in mice compared to control. On the other hand, the author found that aspartame did not decrease the mitotic index and did not induce a significant increase of Sister Chromatid Exchange (SCEs) compared to the control. Renuzguillari et al. (2004) studied the genotoxic effects of aspartame on human lymphoocytes in vitro using chromosomal aberration test, SCE test and micronucleus test. They found that aspartame induced a significant increase of chromosomal aberrations. They reported that aspartame showed cytotoxic effect by decreasing the mitotic index at all concentration and treatment periods. Mukhopadhyay et al. (2000) evaluated the effect of blends of aspartame and aceulamine-K on induction of chromosomal aberration in bone marrow cells of male mice. The authors observed increase in the percentage of cells with chromosomal aberrations with increasing doses of the two sweeteners.

The present study showed that administration of aspartame to mothers of the 1st and 2nd treated subgroups increased the frequency of numerical chromosomal aberrations in their bone marrow cells. Also, our study showed increased the frequency of numerical chromosomal aberrations in the liver and bone marrow cells of the offspring of the 1st and 2nd treated subgroups, respectively. The present result is supported by Nakao et al. (2003), who tested the possibility of micromolar formaldehyde, a metabolite of methanol derived from aspartame, exerts cytotoxicity. Starchan and Read (2010) explained the occurrence of numerical aberrations through two main mechanisms.

In the present study, administration of aspartame to mothers and their offspring of the 1st and 2nd treated groups induced highly significant increase of the mean percentage of DNA fragmentation in their liver cells compared to the control mothers group. These results suggested that aspartame had a genotoxic risk. This finding is in agreement with some previous studies. Karikas et al. (1998) measured the direct molecular interaction of aspartame and its metabolites with DNA as an indicator of possible carcinogenic potential in an in vitro model. They observed measurable, dose-related molecular interaction with DNA by aspartame, as well as
its components aspartic acid, phenylalanine and methanol. Trocho et al. (1998) suggested that the regular intake of aspartame may result in the progressive accumulation of formaldehyde adducts responsible of functional alteration of proteins and of DNA mutations, effects leading to autoimmunity, cell death or malignant transformation.

Many authors described the mechanism through which aspartame could induce DNA fragmentation and chromosomal aberrations. Abhilash et al. (2011) mentioned that a small amount of aspartame significantly increased the plasma methanol levels. The authors stated that methanol intoxication was associated with increased levels of free radicals production. Seoane et al. (1995) mentioned that the free radicals were responsible for the induction of cellular DNA damage that leads to formation of chromosomal aberrations. Also, Alleva et al. (2011) stated that aspartame is a potential angiogenic agent that can induce ROS production. ROS stimulate induction of number of cytokines and growth factors as it enhance interleukin 6, vascular endothelial growth factor and their soluble receptors release from the endothelial cells.

The present study showed that withdrawal of aspartame in the 3rd treated group (mothers and offspring) compared to the control group showed highly significant reduction in their body weight. However, the percentage of this reduction was the least among the other treated groups. In addition, withdrawal of aspartame improved the histological changes of the liver as the hepatic parenchyma mostly restored its normal architecture. Moreover, chromosomal aberrations study clarified that withdrawal of aspartame in the mothers showed decrease of the total structural chromosomal aberrations in their bone marrow cells. Quantitation of DNA fragmentation study clarified that withdrawal of aspartame in the mothers showed highly significant decrease of the mean percentage of DNA fragmentation in their liver cells compared to the mothers of the 1st and 2nd treated groups.

These results coincide with many researchers. Junqueira and Mescher (2010) mentioned that the liver has a strong capacity for regeneration despite its slow rate of cell renewal. The loss of hepatic tissue from the action of toxic substances triggers a mechanism by which the remaining healthy hepatocytes begin to divide, in a process of compensatory hyperplasia, continuing until the original mass of tissue is restored. Ross and Pawlina (2011) mentioned that the cells in the periportal zone of the hepatic lobule are the first to receive oxygen, nutrients and toxins from the sinusoidal blood and the first to regenerate.

CONCLUSION

In conclusion, the consumption of aspartame leads to histopathological lesions in the liver and alterations of the genetic system in the liver and bone marrow of mother albino rats and their offspring. These toxicological changes were directly proportional to the duration of its administration and improved after its withdrawal.

REFERENCES


