Light and Electron Microscopic Study on the Effect of Aspartame on the Cerebellar Cortex of Male Albino Rat

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ABSTRACT

Introduction: Aspartame (ASP) is the most widely used artificial sweeteners entering in the component of many foods and beverages. There was a great matter of controversy about the effect of aspartame on body cells.

Aim of the Work: Was to evaluate the effect of aspartame on the structure the cerebellar cortex in rats.

Materials and Methods: Fifteen male adult albino rats were used. The animals were divided into three groups, each containing five rats. Group I served as control. Group II included the animals which received ASP orally in a dose of 250 mg/kg daily for 8 weeks. Group III included the animals which received the same dose of ASP for 8 weeks, then left for recovery for 4 weeks after stoppage of ASP. The specimens of cerebellum were processed for H&E, Golgi-cox, immunohistochemical staining for glial fibrillary acidic protein (GFAP) and caspase-3 and electron microscopic study. Morphometric and statistical study were done to measure area percentage for GFAP immuno-stained sections.

Results: Group II (ASP) sections of the cerebellar cortex showed that some of the Purkinje cells lost their characteristic pyriform shape, had condensed nuclei with affection of their processes. Some vacuolations were seen in the granular and the molecular layer. There was a significant increase in area percentage of the GFAP immuno-staining sections. Few Purkinje cells appeared positive to caspase-3 immuno-reaction. Electron microscopic examination revealed affection of some Purkinje cell nucleus and organelles, of granule cells and of neuronal processes. Group III (Recovery) sections showed incomplete restoration of the normal structure of the cerebellar cortex.

Conclusion: It was concluded that aspartame consumption had a harmful effect on the structure of the cerebellar cortex of the rat. The period of 4 weeks stoppage of aspartame administration was not enough to get a complete recovery.

Key Words: Aspartame, cerebellal cortex, rat, electron microscope.

INTRODUCTION

Artificial sweeteners are used frequently nowadays to reduce sugar consumption and to decrease caloric intake in healthy persons as well as in diabetic patients. Aspartame (ASP) is the most widely used artificial sweeteners. It is a methyl ester of a dipeptide (L-aspartyl-L-phenylalanine methyl ester). It enters in the component of many foods and beverages. It had about 200 fold higher sweetness than sucrose.

Aspartame is hydrolyzed in the intestine to its components: phenylalanine, aspartate and methanol. Each one of these components are toxic and affect different body organs mainly the central nervous system. Fifty percent of ASP is comprised of phenylalanine, which could cross the blood-brain barrier and act as a precursor of catecholamines in the brain. Forty percent of ASP is comprised of aspartate (or aspartic acid), which is an excitatory neurotransmitter. Methanol, forming 10% of the broken down products of ASP, is a toxic compound converted in the liver to formaldehyde which is neurotoxin and carcinogen.

There was a great matter of controversy about the effect of aspartame on body cells. Some investigators suggested that the rise in human brain tumors may be related to worldwide ASP use. Others showed that ASP consumption might result in seizures, memory loss, dizziness, headaches and behavioral changes. On the contrary, some authors reported that the studies on ASP did not support an association between aspartame and cancer in any tissue and that ASP is safe.

As most of the previous studies were concerned about the effect of ASP on memory, learning and behavioral changes. This study would investigate the part of the central nervous system concerning with the unconsciousness. So, the aim of this study was to evaluate the effect of aspartame on the structure the cerebellar cortex in the adult male albino rats.
MATERIALS AND METHODS

Fifteen male adult albino rats, weighing about 200 gm, were used in this study. They were housed in standard cages and had free access to water and standard diet at the medical research center in Ain Shams University. The animals were divided into three groups as the following:

**Group I: (Control Group):** Which contained five rats, serving as control animals.

**Group II: (ASP Group):** Including five rats, which received daily 250 mg/kg of aspartame (ASP) dissolved in distilled water and given orally to the animals by intra-gastric tube for 8 weeks. Aspartame tablets, each one containing 20 mg, were obtained from Al-Ameriya Pharma Company. This dose used is corresponding to the human dose, which is 40-50 mg/kg per day, after species factor correction requiring 5-6 times higher dose than man, as rat metabolizes ASP faster than human.  

**Group III: (Recovery Group):** Including five rats, which received daily 250 mg/kg body weight of aspartame (ASP) orally as the previous group for 8 weeks. Then, the rats were left for 4 weeks without ASP administration as recovery animals.

At the end of the experiment, the rats were sacrificed by decapitation. The cerebellum was removed immediately and cut to three parts to be subjected to the followings:

**Light Microscopic Study:**

1. Part of the cerebellum was cut and fixed in 10% neutral buffered formalin and processed for light microscopic study to get paraffin sections of 5 µm thickness. Sections were stained with:
   1. Haematoxylin and Eosin (H&E).
   2. Immunohistochemical staining (purchased from Lab vision, USA) for:
      - Gliarial fibrillary acidic protein (GFAP) of glial cells.
      - Caspase-3 to detect apoptosis.

Serial paraffin sections of 5 µm thick were deparaffinized and dehydrated, including the positive control sections (retina in GFAP and tonsil in caspase-3). The endogenous peroxidase activity was blocked with 0.05% hydrogen peroxide in absolute alcohol for 30 minutes. The slides were washed 5 min in phosphate buffered saline (PBS) at pH=7.4. To unmask the antigenic sites, sections were put into 0.01M citrate buffer (PH=6) in the microwave for 5 min. The slides were incubated in 1% bovin serum albumin dissolved in PBS for 30 min at 37°C in order to prevent the non specific background staining. Two drops of ready to use primary antibody were applied to sections, except for negative control, then they were incubated for one hour and half at room temperature. On half the number of the sections GFAP was applied, while caspase-3 was applied to the remaining half of the sections. The slides were rinsed with PBS, then incubated for one hour with anti-mouse immunoglobulins (secondary antibody) conjugated to peroxidase labeled dextran polymer (DAKO, Denmark). In order to detect the reaction, the slides were incubated in 3,3-diaminobenzidine (DAB) for 15 min. The slides were counterstained by Mayer’s Haematoxylin, then dehydrated, cleared and mounted by DPX.

2. Another part of the cerebellum was cut and fixed in Golgi-cox solution for 2 months to stain the neurons and their processes. Then, specimens were processed as the usual paraffin technique. Ten micron paraffin sections were cut and put in 10% ammonia solution for one hour. Sections were dehydrated, cleared and mounted by DPX.

**Electron Microscopic Study:**

The third part of the cerebellum was cut in small pieces of 1mm² size and fixed in 2.5% glutaraldehyde for 24 hours. Specimens were washed in 0.1 M phosphate buffer at 4°C, then post fixed in 1% osmium tetroxide at room temperature. Specimens were dehydrated in ascending grades of ethyl alcohol, then embedded in Epon resin. Semithin sections (1µm) were stained with toluidine blue in borax and examined with light microscope. Ultrathin sections (50 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate. Specimens were examined and photographed with JEM 1200 EXII transmission electron microscope in Faculty of Science, Ain Shams University.

**Morphometric and Statistical Study:**

The area percentage of the immuno-reactive glial cells stained by GFAP was calculated from the total area percentage of the cerebellar cortex. The number of Purkinje cells with positive brownish caspase-3 immuno-reaction per high power field was calculated. Five fields from three serial sections of each rat from each group were examined by high power lens (X40), using of the image analyzer Leica Q500MC program in Histology Department, Faculty of Medicine, Ain Shams University. Mean and standard deviation (SD) of area percentage and number of positive caspase-3 immuno-reaction Purkinje cells were statistically analyzed using t-test and one way ANOVA with post-hoc test of SPSS 17 was done to calculate P value. The calculations were considered significant if P < 0.05.
RESULTS

Light Microscopic Results:

In H and E examined sections of the control group, cerebellum appeared as irregular folia formed of external gray matter (cerebellar cortex) and internal white matter. The cerebellar cortex was formed of three layers: outer molecular layer, middle Purkinje cell layer and inner granular layer (Fig. 1). The molecular layer was formed mainly of fibers with few cells (stellate and basket cells). Purkinje cells of the middle layer, arranged in one row, appeared pyriform in shape with deeply-stained basophilic cytoplasm and large round, central and vesicular nuclei. Granular layer showed large number of small deeply-stained cells, which are the granular cells, with non-cellular areas in-between the cells representing the cerebellar islands (Fig. 2).

In Golgi-cox stained sections of the control group, Purkinje cells appeared pyriform in shape with multiple branched dendrites (Fig. 3).

Immunohistochemical stained sections for GFAP of the same group showed few brownish star-shaped cells in the granular layer in-between cells and islands. Elongated cells and their processes with faint brownish immuno-reaction appeared in the molecular layer (Fig. 4). Whereas in the Caspase-3 immunohistochemical stained sections, cerebellar cortex revealed no brownish immuno-reaction in the three layers (Fig. 5).

In H and E examined sections of ASP group, vacuolations (spongiosis) appeared between cells of the granular layer and also were seen between fibers of the white matter (Fig. 6). Most of the Purkinje cells appeared deformed and shrunken loosing their characteristic pyriform shape. Some of them showed condensed nuclei. Halo of empty spaces appeared around the Purkinje cells. Some vacuolations were seen between cells of the granular layer and in the molecular layer (Fig. 7).

In Golgi-cox stained sections of the ASP group, Purkinje cells appeared shrunken with beaded dendrites (Fig. 8).

Immunohistochemical stained sections for GFAP of the ASP group showed increased brownish immuno-reaction of the star-shaped cells in the granular layer in-between cells and cerebellar islands and in the Purkinje cell layer with apparent increase in their number and size. Deeply brownish immunoreaction appeared in elongated cells and their processes in the Purkinje and molecular layers (Fig. 9). Few Purkinje cells showed brownish cytoplasmic immuno-reaction for caspase-3, detecting cell apoptosis (Fig. 10).

In the recovery group, the H and E stained sections revealed vacuolated areas in the granular layer between cells and in the white matter between fibers (Fig. 11). Purkinje cells regained their pyriform shape and their nuclei appeared vesicular (Fig. 12).

Golgi-cox stained sections of the recovery group showed that Purkinje cells regained their characteristic shape, but dendrites appeared slightly beaded (Fig. 13).

In the immunohistochemical stained sections for GFAP, the star-shaped cells in the granular layer of the recovery group appeared less in immuno-reaction and number than the ASP group but more than the control group. Elongated cells and their processes appeared also with less immuno-reaction in the molecular layer compared to the ASP group (Fig. 14). Whereas in the Caspase-3 immunohistochemical stained sections, the immuno-reaction was seen in the cells of three layers of the cerebellar cortex, in the glial cells between the small granule cells, in few of Purkinje cells and also in glial cells present in molecular layer (Fig. 15).

Electron Microscopic Results:

In the ultra-structural examination of the control group, Purkinje cells appeared large in size surrounded by different nerve fibers and cells. Their nuclei were large, ovoid with irregular nuclear membrane and apparent nucleoli. Cisternea of rER, ribosomes, mitochondria, Golgi complex and some lipid granules appeared in the cytoplasm (Fig. 16). Granule cells appeared small cells with large and slightly darker nuclei with thin rim of cytoplasm, while Golgi type II cells were seen with larger diameter and lighter nuclei. These cells surrounded the Mossy rosettes containing many mitochondria and synaptic vesicles (Fig. 17). Myelinated fibers could be detected between cells. They contained mitochondria and synaptic vesicles. Microtubules were also seen in the nerve fibers (Fig. 18).

In ASP group, Purkinje cells showed smaller nuclei with more condensed chromatin. Vacuolations and dilated Golgi complex were seen. Some mitochondria appeared with destructed cristae. Cells were surrounded by empty spaces (Fig. 19). In the granular layer, many granule cells showed less condensed nuclei and star-shaped cells appeared with condensed nuclei (Fig. 20). Mitochondria in the nerve fibers and dendrites appeared with more condensed matrix. Some myelinated fibers showed splitting of their myelin sheath (Fig. 21).

Purkinje cells in the recovery group showed non-dilated cisternea with many mitochondria. Their nuclei appeared lighter with more extended chromatin (Fig. 22). Granule cells still appeared with light nuclei showing some indentation. No star shaped cells with condensed nuclei could be detected in the granular layer (Fig. 23). Mitochondria showed slightly condensed matrix in the nerve fibers. Splitting of the myelin sheath also could be seen (Fig. 24).
Morphometric and statistical results:

Table (1) and Histogram (1) showed the mean ± SD of the area percentage of GFAP immuno-stained sections of different groups. Table (1*) showed that in ASP group, there was a significant increase in the area percentage of the GFAP stained cells in comparison with those of the control and recovery group. While, the recovery group revealed a significant decrease in comparison to the ASP group and a significant increase in comparison with the control group.

Table 1: Showing the mean ±SD of the area percentage of GFAP immuno-stained cerebellar cortex sections in different groups:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean(%)</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>3.32</td>
<td>1.31</td>
</tr>
<tr>
<td>Group II (ASP)</td>
<td>11.07</td>
<td>2.86</td>
</tr>
<tr>
<td>Group III (Recovery)</td>
<td>6.90</td>
<td>1.79</td>
</tr>
</tbody>
</table>

SD = standard deviation

Histogram 1: Showing the mean of the area percentage of GFAP immuno-stained cerebellar cortex sections in different groups.

Table 1*: Showing the significance of difference in mean of the area percentage of GFAP immuno-stained sections in different groups using one way ANOVA with post-hoc test.

<table>
<thead>
<tr>
<th>(I) groups</th>
<th>(J) groups</th>
<th>Mean Difference (I-J)</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Group II</td>
<td>-7.76000</td>
<td>.000</td>
<td>S</td>
</tr>
<tr>
<td>Group I</td>
<td>Group III</td>
<td>-3.58000</td>
<td>.019</td>
<td>S</td>
</tr>
<tr>
<td>Group II</td>
<td>Group I</td>
<td>7.76000</td>
<td>.000</td>
<td>S</td>
</tr>
<tr>
<td>Group II</td>
<td>Group III</td>
<td>4.18000</td>
<td>.008</td>
<td>S</td>
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<tr>
<td>Group III</td>
<td>Group I</td>
<td>3.58000</td>
<td>.019</td>
<td>S</td>
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<tr>
<td>Group III</td>
<td>Group II</td>
<td>-4.18000</td>
<td>.008</td>
<td>S</td>
</tr>
</tbody>
</table>

P value < 0.05 is significant S = significant

Table (2) and Histogram (2) showed the mean ± SD of the number of Purkinje cells with positive brownish caspase-3 immuno-reaction per high power field of different groups. Table (2*) showed that in ASP group, there was a significant increase in the number of Purkinje cells with positive brownish caspase-3 immuno-reaction per high power field in comparison with those of the control and recovery group. While, the recovery group revealed a significant decrease in comparison to the ASP group and a non significant increase in comparison with the control group.

Table 2: Showing the mean ± SD of the number of Purkinje cells with positive brownish caspase-3 immuno-reaction per high power field of different groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean of number in μm²</th>
<th>± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>.5</td>
<td>.71</td>
</tr>
<tr>
<td>Group II (ASP)</td>
<td>2.0</td>
<td>1.25</td>
</tr>
<tr>
<td>Group III (Recovery)</td>
<td>1.1</td>
<td>.74</td>
</tr>
</tbody>
</table>

SD = standard deviation

Histogram 2: Showing the mean of the number of Purkinje cells with positive brownish caspase-3 immuno-reaction per high power field of different groups.

Table 2*: Showing the significance of difference in mean of the number of Purkinje cells with positive brownish caspase-3 immuno-reaction per high power field of different groups using one way ANOVA with post-hoc test.

<table>
<thead>
<tr>
<th>(I) group</th>
<th>(J) group</th>
<th>Mean Difference (I-J)</th>
<th>P value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
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<td>Group I</td>
<td>Group II</td>
<td>-1.50000*</td>
<td>.001</td>
<td>S</td>
</tr>
<tr>
<td>Group I</td>
<td>Group III</td>
<td>-.60000</td>
<td>.161</td>
<td>NS</td>
</tr>
<tr>
<td>Group II</td>
<td>Group I</td>
<td>1.50000*</td>
<td>.001</td>
<td>S</td>
</tr>
<tr>
<td>Group II</td>
<td>Group III</td>
<td>.90000*</td>
<td>.040</td>
<td>S</td>
</tr>
<tr>
<td>Group III</td>
<td>Group I</td>
<td>.60000</td>
<td>.161</td>
<td>NS</td>
</tr>
<tr>
<td>Group III</td>
<td>Group II</td>
<td>-.90000*</td>
<td>.040</td>
<td>S</td>
</tr>
</tbody>
</table>

P value < 0.05 is significant S = significant NS = non significant

Fig. 1: A photomicrograph of a section of control group showing a cerebellum folium formed of external gray matter [cerebellar cortex] (C) and internal white matter (W). The three layers of cerebellar cortex are seen: outer molecular layer (M), middle Purkinje cell layer (P) and inner granular layer (G). H&E X 100
Light and Electron Microscopic Study on the Effect of Aspartame on the Cerebellar Cortex of Male Albino Rat

Fig. 2: A photomicrograph of a section of control group showing molecular layer (M) formed mainly of fibers with few cells. Purkinje cells (↑) are arranged in one row and appear pyriform in shape with deeply-stained basophilic cytoplasm and large round, central and vesicular nuclei. Granular layer showed large number of small deeply-stained cells with cerebellar islands (C) in-between. H&E X 640

Fig. 3: A photomicrograph of a section of control group showing Purkinje cells pyriform in shape with multiple branched dendrites (↑). Golgi-cox X 640

Fig. 4: A photomicrograph of a section of control group showing few brownish star-shaped cells (►) in the granular layer. Elongated cells and their processes appear in the molecular layer (M) with faint brownish immuno-reaction. GFAP X 640

Fig. 5: A photomicrograph of a section of control group showing no brownish immuno-reaction in the three layers of the cerebellar cortex. Caspase-3 X 640

Fig. 6: A photomicrograph of a section of ASP group showing vacuolations (spongiosis) (►) between cells of the granular layer (G) and between fibers of the white matter (W). H&E X 100

Fig. 7: A photomicrograph of a section of ASP group showing deformed and shrunken Purkinje cells (↑) with condensed nuclei. Halos of empty spaces appear around the Purkinje cells. Notice vacuolations (►) between cells of the granular layer (G) and in molecular layer (M). H&E X 640
Fig. 8: A photomicrograph of a section of ASP group showing shrunken Purkinje cells (↑) with beaded dendrites. Golgi-cox X 640

Fig. 9: A photomicrograph of a section of ASP group showing apparent increase in number and size of star-shaped cells (►) in the granular layer and Purkinje cell layer with increase in their brownish immuno-reaction. Elongated cells and their processes appear in the molecular layer (M) with Deep brownish immunoreaction. GFAP X 640

Fig. 10: A photomicrograph of a section of ASP group showing brownish cytoplasmic immuno-reaction in few Purkinje cells (↑) detecting cell apoptosis. Caspase-3 X 640

Fig. 11: A photomicrograph of a section of recovery group showing vacuolations (spongiosis) (►) between cells of the granular layer (G) and between fibers of the white matter (W). H&E X 100

Fig. 12: A photomicrograph of a section of recovery group showing that Purkinje cells (↑) regain their pyriform shape and vesicular nuclei. H&E X 640

Fig. 13: A photomicrograph of a section of recovery group showing pyriform shape of Purkinje cell and dendrites appear slightly beaded (↑). Golgi-cox X 640
Fig. 14: A photomicrograph of a section of recovery group showing less brownish immuno-reaction and number of star-shaped cells (►) in the granular layer and elongated cells in molecular layer (M) compared to the previous group. GFAP X 640

Fig. 15: A photomicrograph of a section of recovery group showing one Purkinje cell (↑) with brownish immuno-reaction as well as in the glial cells between the small granular cells (►) and also in glial cells present in molecular layer (M). Caspase-3 X 640

Fig. 16: An electron micrograph of control group showing a Purkinje cell with large, ovoid nucleus (N) and apparent nucleolus (↑). Cisternae of rER (R), mitochondria (m), Golgi complex (►) and some lipid (L) granules appear in the cytoplasm. TEM X 6000

Fig. 17: An electron micrograph of control group showing structure of granular layer. Small granule cells (G) appear with slightly darker nuclei and thin rim of cytoplasm. Golgi type II cells (II) appear with larger diameter and lighter nuclei. Mossy rosettes (R) are seen containing many mitochondria and synaptic vesicles. TEM X 4000

Fig. 18: An electron micrograph of control group showing myelinated fibers with mitochondria (↑). Notice microtubules (M) in nerve fibers. TEM X 15,000

Fig. 19: An electron micrograph of ASP group showing a shrunken Purkinje cell with smaller nucleus with more condensed chromatin (N) and apparent nucleolus (↑). Dilated Golgi complex (►) and mitochondria with destructed crista (m) appear in the cytoplasm. Notice empty spaces surrounding the cell. TEM X 6000
DISCUSSION

Artificial sweeteners are nowadays essential food additives. Aspartame (ASP) is the most frequent used one. Once entering the body, ASP was hydrolyzed to form phenylalanine, aspartic acid and methanol. Each component might be the result of different neurological and behavioral symptoms produced after ASP consumption. This study aimed to evaluate effect of aspartame on the structure of the cerebellar cortex of male albino rat.

In ASP group of the current study, H and E stained sections revealed deformed Purkinje cells with condensed nuclei. The ultra-structure of these cells seen by the electron microscope showed affected Purkinje cells. These cells appeared shrunken with condensed chromatin in their nuclei. The cytoplasm showed dilated Golgi complex, vacuolations and destructed cristae of mitochondria. These changes in Purkinje cells revealed affection of the main cells of the cerebellar cortex as they...
are the only cells of the cerebellum to send information to the outside\textsuperscript{12}. These findings could be explained as phenylalanine, comprising 50% of aspartame metabolism when entering the body, could cross the blood brain barrier by competition on the binding sites for transporting other essential amino acids. Phenylalanine decreased catecholamines neurotransmitter level\textsuperscript{13}. Another released component was the aspartic acid, also known as aspartate, which comprised 40% of aspartame. Aspartic acid was an excitatory neurotransmitter in the central nervous system. It increased the depolarization at the postsynaptic membrane. This might cause rapid firing of the neurons\textsuperscript{5}. Increased level of these substances could change the activity of the brain and alter its enzymes\textsuperscript{14}. Other investigators\textsuperscript{15} denoted that aspartic acid caused a significant change in L-glutamate binding sites in the brain, which was dose-dependently inhibition of L-glutamate to bind to its specific receptors. In this study, Purkinje cells of ASP group appeared deformed and their dendrites were affected and beaded in Golgi-cox stained sections. Using caspase-3 immunostaining, few Purkinje cells appeared with brownish immuno-reaction denoting their apoptosis and there was a significant increase in their number compared to the control group. These findings are in agreement with some investigators\textsuperscript{6} who mentioned that aspartic acid might excite the brain cells till death. Some authors\textsuperscript{16} explained the neuronal death as it might be triggered by excitotoxins. Excessive stimulation of the excitatory glutamate receptors by glutamate receptor agonist (like aspartic acid released from ASP) could increase ion movement across the cell membrane. Sequestration of excess calcium into the mitochondria and the endoplasmic reticulum might be harmful on these organelles. As the glutamate receptors were also found in the dendrites, excessive calcium ions could affect them. This could explain the affection of mitochondria in cell processes of the granular layer which appeared in the ultra-structural examination in ASP group. Moreover, the stressed mitochondria released cytochrome c, which is one of the key enzymes in oxidative phosphorylation. This enzyme activated caspase-9 which in turn act on caspase-3 to initiate irreversible phases of apoptosis (programmed cell death)\textsuperscript{17}. Other workers suggested that caspase-3 was responsible for cleavage and inactivation of plasma membrane calcium pump in neurons\textsuperscript{18}.

Aspartate also resembled in structure to the glutamate, which is a transmitter at excitatory synapses in the cerebellum, as cerebellar molecular and granular cell layer, the parallel fiber and mossy fiber synapses\textsuperscript{6}. glutamine was the precursor for releasable aspartate via glutaminase in the parallel and mossy fibers. After release of glutamate and aspartate, they were taken up by the processes of the surrounding glial cells. GABA, the inhibitory neurotransmitter of the cerebellum, was also synthesized from glutamate in the Golgi type II cell terminals and taken up by surrounding glial cells\textsuperscript{19}. These findings could explain why the GFAP immuno-stain in the present study showed increased number, size and immuno-reaction of the glial cells in the three layers of the cerebellar cortex. This increased in glial cells was confirmed by morphometric study showing a significant increase of its area percentage. In the ultra-structural examination, many star-shaped cells appeared in-between cells of the granular layer. These findings of the present study might be in correlation with many authors who reported the high incidence of brain tumors mainly the gliomas in human\textsuperscript{6} and in rats\textsuperscript{20}.

Another component was formed from the hydrolysis of aspartame, which was the methanol. It was converted in the liver to formaldehyde, which is known to be neurotoxin and carcinogen\textsuperscript{6}. This could lead to increase in the metabolizing enzymes of the cerebellum as well as other sites in rat brain\textsuperscript{21,22}. Formaldehyde also attached to the DNA, RNA and proteins of the cells and became difficult to be removed, which might cause breaks in the DNA\textsuperscript{23}. These findings could also explain the affection noticed in the Purkinje cells and cell processes in the present study.

In the present study, the ASP group showed splitting in some areas of the fiber myelin sheath, when examined by the electron microscope. This finding could be explained by some authors who reported that excitotoxins acting at different sites within the central nervous system could strip myelin from fibers and destroying neurons\textsuperscript{24}.

There was controversy with the previous results. Some investigators found no effects of aspartame on motor activity, convulsions\textsuperscript{25}, cognitive, memory and behavioral tasks\textsuperscript{26}. Other authors\textsuperscript{3-27} reported that no association between aspartame consumption and cancer occurrence.

In the current study, a recovery group was performed to evaluate stoppage of aspartame administration for 4 weeks on the structure of the cerebellar cortex. This group showed regain of normal shape and nuclei of the Purkinje cells in H and E and Golgi-cox stained sections. The ultra-structure of these cells revealed less vacuolations and nearly normal mitochondria. These findings denoted restoration of Purkinje cells to their normal structure when the causative substances were removed. Some workers\textsuperscript{28} reported that after cessation of ASP use, a vestibulo-cochlear toxicity was recovered. There was still some affection of the cells as few Purkinje cells showed caspase-3 positive immuno-reaction. But, the morphometric study revealed a significant decrease in the number of positive caspase-3 Purkinje cells compared to ASP group. In the Golgi-cox stained sections, the dendrites of some cells appeared beaded. The mitochondria of the cell processes appeared condensed with splitting in the myelin sheath of some axons on the ultra-structural examination. Some authors\textsuperscript{24} reported that remyelination might occur after recovery of lesion but taking over a period of 3 Months.
The area percentage of GFAP-stained glial cells appeared significantly decreased compared to ASP group, but still significantly increased than the control group. This finding coincided with an author who reported that microglial cells might support the neuronal recovery through release of some cytokines and growth factors29. It seemed that the recovery period was not enough to restore completely the normal structure of the cerebellar cortex.

CONCLUSION

In conclusion, aspartame appeared to have a harmful effect on the structure of the cerebellar cortex of the albino rat. It is recommended to avoid the use of aspartame as much as possible. If it is necessary for use, decrease its dose and did not exceed the daily recommended dose.

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دراسة بالميكروسكوب الضوئي والكيميائي على تأثير الأسبرتام على قشرة المخيخ لدى جرذ الابيض

عبير عبد المحسن عبد الصمد
قسم الهستولوجيا - كلية الطب - جامعة عين شمس

المقدمة: يعتبر الأسبرتام أكثر محلل صناعي مستخدم وهو يدخل في تركيبة كثير من الأغذية والمشروبات. و هناك جدل كبير بشأن تأثير الأسبرتام على مختلف خلايا الجسم.

النهاية من الدراسة: هو تقييم تأثير الأسبرتام على بيئة قشرة المخيخ في الجرذان.

المادة والطريقة: تم استخدام خمسة عشر جرذ أبيض ذكر بالغ، تم تقسيمهم إلى ثلاث مجموعات تحتوي كل منها على خمسة جرذان. المجموعة الأولى تمثل المجموعة الضابطة و قد تكونت المجموعة الثانية من الحيوانات التي أخذت الأسبرتام بالفم بجرعة 250 ملليجرام لكل كيلوجرام يوميا لفترة 8 أسابيع. و قد تكونت المجموعة الثالثة من الحيوانات التي حصلت على نفس جرعة الأسبرتام لمدة 8 أسابيع ثم تركت لمدة 4 أسابيع بدون أسبرتام. و قد تم تجهيز عينات المخيخ لعمل صبغة الهيماتوكسيلين والإيوسين والبركتين-كونكس، الصبغة الكيميائية المناعية للبروتين الليفي الحمضي للخلايا الدبقية وكسبيرز-3، و كذلك للفحص بالميكروسكوب الإلكتروني. و قد تم عمل دراسة قياسية بالكمبيوتر و دراسة إحصائية لقياس نسبة مساحة الصبغة المناعية للبروتين الليفي الحمضي للخلايا الدبقية.

النتائج: بفحص مقاطع قشرة المخيخ في المجموعة الثانية تبين أن بعض خلايا بركنجي فقدت شكلها الكمثرى المميز و احتوت على ألوية مكثفة و قد تأثرت النتوءات. و قد لوحظ بعض التجاوز في الطبقة المحببة والجزيئية. و قد أزدادت نسبة المساحة للصبغة المناعية للبروتين الليفي الحمضي للخلايا الدبقية زيادة ذو دلالة إحصائية. و قد أظهرت قليل من خلايا بركنجي صبغة مناعية إيجابية للكسبيرز-3. و قد أوضح الميكروسكوب الإلكتروني تأثير في النواة و الجذور العصبية في خلايا بركنجي و في الخلايا المحببة وكذلك في الألياف العصبية. أما في المجموعة الثالثة، فقد أوضحت المقاطع عدم العودة الكاملة إلى البنية الطبيعية لقشرة المخيخ.

الخاتمة: لقد استخلص أن استخدام الأسبرتام له تأثير ضار على بيئة قشرة المخيخ في الجرذ. و أن مدة التوقف 4 أسابيع عن استخدام الأسبرتام لم تكن كافية للشفاء التام.