NEUROTOXIC EFFECT OF ASPARTAME ON THE SCIATIC NERVE OF ADULT MALE ALBINO RAT AND THE POSSIBILITY OF SPONTANEOUS RECOVERY: LIGHT AND ELECTRON MICROSCOPIC STUDY

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ABSTRACT:

Background: *Aspartame is considered the most widely used no-caloric artificial sweetener. It is used in a variety of food products and beverages. However, the consumption of aspartame has been controversial as several studies have reported an association between its use and many serious diseases.*

Aim of the work: *The aim of the present study was to evaluate the potential neurotoxic effect of long duration aspartame consumption on the sciatic nerve of the adult male albino rats and the possibility of spontaneous recovery following cessation of its administration.*

Material and methods: *Thirty adult male albino rats were used in this study, aged from 6-8 months, weighting 180 -200 gm. Rats were randomly divided into three groups: Group I: ten rats that received nothing except food and water, Group II: ten rats that received aspartame (250 mg/kg/d) for 12 weeks and Group III: ten rats that received aspartame as in group II then left for four weeks to recover.*

Results: *The present work revealed that aspartame induced histological changes of rats' sciatic nerves in the form of irregular nerve structure with wide separation of myelinated nerve fibers and dark elongated Schwann cells. Ultrastructural examination of group II showed separated myelin lamellae and excessive in folding with myelin loops formation. The recovery group showed histological improvement when compared to group II, yet it didn't reach completely to the histological picture of the control group.*

Conclusion: *The results supported the neurotoxic effect of aspartame on rats' sciatic nerves when consumed regularly for a long period and proved that the spontaneous recovery wasn't complete.*

Keywords*: Aspartame, Sciatic nerve, Recovery*

INTRODUCTION:

Aspartame (L-aspartyl- L-phenylalanine methyl ester) is a non-caloric; non-nutritive artificial sweetener which is widely consumed worldwide. It is added to a variety of beverages and food products such as breakfast cereals, chewing gum, and yoghurt products to attenuate the increasing rates of both obesity and diabetes mellitus and it is also widely prescribed in weight reduction regimen[1-3]. In addition, it is used in manufacturing some pharmacological products such as chewable multi-vitamins and cough drops[2,4].

Although the Food and Drug Administration (FDA) has approved the consumption of aspartame, yet, its use has been controversial as it has been associated with

adverse effects on different organs such as liver and brain[3,5]. Aspartame is metabolized in the body to phenylalanine (50%), methanol (10%) and aspartate (40%). Each metabolite could produce toxic effects on the nervous system[5]. Aspartate is a brain neurotransmitter. However, it is present in the extracellular fluid in a very small concentration. When the concentration exceeds certain level, the cells start a process of excitotoxicity leading to delayed cell death, which means that the cells are excited to death[6].

It has been proved that aspartame can induce toxicity to several body tissues. Previous experimental studies suggested an association between the use of aspartame and many health problems such as increasing the risk of type II diabetes[7], nephrotoxicity[8], hepatotoxicity[9], preterm delivery[10], and the induction of structural changes in the parotid glands[11]. In addition, it was reported that aspartame could increase the risk of cancer development[12].

As regard the neurotoxic effect of aspartame, most of the experimental studies were concerned about central nervous system toxicity. Previous studies suggested that aspartame intake led to neurological and behavioral changes in humans[13]. In addition, aspartame consumption was proved to affect the structure of the cerebral[14] and the cerebellar cortices[4&15]. Moreover, it has been reported that aspartame consumption affects the memory, learning and behavior[16].

Albino rat sciatic nerve histologically mimics the human sciatic nerve except for the difference in size and connective tissue density, that's why it is the most used nerve in experimental studies on peripheral nerves[17&18]. The sciatic nerve is formed of myelinated, unmyelinated nerve axons and two types of Schwann cells (myelinating and non-myelinating). The plasma membrane of myelinating Schwann cell, concentrically wraps around nerve axon forming the myelin sheath that increases the conduction velocity along the axon and insulates the axon. The non-myelinating Schwann cells provide trophic support to the unmyelinated axons without myelination[19].

To our knowledge, by reviewing the literature, very few studies were concerned about studying the neurotoxic effect of aspartame on the peripheral nerves.

AIM OF THE WORK:

The objective of this work was to focus on the histological changes induced by long term aspartame consumption on the sciatic nerve of the adult male albino rat and the possibility of spontaneous recovery following cessation of its administration.

MATERIAL AND METHODS:

- **Chemicals:**

Aspartame was purchased in the form of pure powder from (Sigma–Aldrich chemical, St. Louis, USA).

- **Animals:**

Thirty adult male albino rats were used in the experimental study, aged from six to eight months, weighting 180-200 gm, they were obtained and locally bred at the animal house of the medical research center (Faculty of Medicine, Ain-Shams University). Rats were housed in medium sized metal cages at a room temperature with regular dark/light cycles and good ventilation. Free diet and water access were allowed, all rats were kept under the same circumstances throughout the experiment. The experiment followed the guidelines of Committee of Animal Research Ethics (CARE).

Experimental Protocol:

Rats were equally divided into three groups:

Group I (Control group): included ten rats that received nothing except food and water.

Group II (Aspartame-treated group): included ten rats that received aspartame at a dose of 250 mg/kg body weight /day[20]. The calculated dose was dissolved in 2ml distilled water and given orally by gastric tube for 12 weeks. This dose was corresponding to the acceptable daily dose approved by the FDA which is 40 - 50 mg/kg/d for human [21]. Rats require five times higher dose than humans, as rats metabolize aspartame faster than humans[22].

Group III (Recovery group): included ten rats that received aspartame as in group II. Then, rats were left for four weeks after stopping aspartame administration to recover before they were sacrificed.

By the end of the experimental period, rats of each group were anesthetized with diethyl ether, and the distal part of sciatic nerves of all rats were carefully dissected and processed for microscopic examination.

Specimens preparation and staining

Sciatic nerve was fixed in 10% buffered formalin, processed for preparation of paraffin blocks, the blocks were cut transversely at 5µm and stained by Hematoxylin and Eosin [23].

For GFAP immunohistochemical study avid in-biotin peroxidase technique was used. Paraffin sections (5µm) sections underwent deparaffinization and hydration. The specimens were incubated with the primary antibody (1:100 monoclonal mouse anti GFAP) at 4°C for 18-20hours.The next step was washing and incubating the slides with biotinylated secondary antibodies and then with avid in biotin complex. Lastly, sections were counterstained with hematoxylin before being mounted[24].GFAP positive cells (Non myelinating Schwann cells in peripheral nerves) appear as brown dots [25].

Preparation of semithin and ultrathin sections:

Sciatic nerve specimens were fixed immediately in 2.5 % glutaraldehyde. Fixed tissue samples were washed with phosphate buffer and fixed in 1 % osmium tetra oxide. After alcohol dehydration in ascending grades, they were embedded in epon blocks, semithin sections 1µm thick were cut transversely by ultra-microtome, picked up on a gelatinized glass slides and stained with toluidine blue[26]. For sciatic nerve ultrathin sections, 50 nm thick sections were cut using the same ultra-microtome then sections were picked on uncoated mesh copper grids and stained with uranyl acetate and lead citrate[27].

Stained paraffin and semithin sections were examined and photographed using Olympus 268M light microscope equipped with an automatic photo micrographic camera system. While, the ultrathin sections were examined and photographed using Jeol-Ex1010 transmission electron microscope at the regional center of mycology and biotechnology (Al-Azhar University).

Image analysis:

Morphometric analysis was carried out on toluidine blue stained semithin sections of sciatic nerve, using Image j software on a computer in anatomy Department, Faculty of Medicine, Ain Shams University. The computer was connected to Olympus microscope equipped with a digital camera. Six randomly chosen non overlapping fields in six sections obtained from six different animals from the same group were used for measuring the thickness of myelin sheath of the myelinated nerve fibers of the three groups. Pixels were calibrated for actual measurements using a stage micrometer. The magnification used was 1000X with an objective lens 100X.

Statistical analysis:

Data analysis was performed using PSPP freeware with one-way ANOVA and Bonferroni Post Hoc test to detect the significance between every two groups. Results were considered highly significant when P-value $\leq$ 0.001, significant when P-value $\leq$ 0.05 and non-significant when P-value > 0.05.

RESULTS:

Histological Results:

Group I (Control group):

Light microscopic examination of Hx. & E. stained sections of the sciatic nerves of the control group showed densely packed nerve fibers arranged in bundles (fasciculi) of different sizes. Each fasciculus was surrounded by a thin connective tissue (perineurium) and all the fasciculi were enveloped by a thick vascular connective tissue (epineurium). The nerve fibers were mainly myelinated nerve fibers of variable diameters. Each myelinated nerve fiber was formed of a central axon surrounded by a myelin sheath. Schwann cells nuclei were hardly noticed between the nerve fibers (Figs.1-3).

Examination of the GFAP immune-histochemical stained sections revealed weak reaction of scattered GFAP positive cells between the nerve fibers (Fig. 4).

Semithin sections stained with toluidine blue showed that the nerve fibers were mainly of myelinated type of variable diameters and regular myelin sheath of variable thickness with minimal in folding. Minimal loose connective tissue (endoneurium) was noticed between the nerve fibers (Figs. 5 & 6).

Electron microscopic examination showed myelinated axons enveloped by uniform thick and compact lamellar myelin sheath with minimal invaginations. Also, some pale stained unmyelinated axons were observed arranged in groups close to the myelinating axons. Myelinating Schwann cell appeared having large oval nucleus and each cell was surrounding one myelinated axon. While, multiple unmyelinated axons were in vaginating the non-myelinating Schwann cell that showed small pale stained oval to triangular nucleus (Figs. 7,8).

Group II (Aspartame-treated group):

Light microscopic examination of Hx. & E. stained sections of group II showed irregular sciatic nerve structure in the form of thick irregular epineurium, hardly detected perineurium and wide separation of myelinated nerve fibers with loss of some central axons. Most of Schwann cells showed dark elongated nuclei (Figs. 9 &10).

Examination of the GFAP immune-histochemical stained sections revealed an apparent moderate increase in GFAP positive cells (Fig. 11).

Semithin sections stained by toluidine blue clarified that most of the myelinated fibers appeared with changes in their myelin sheath in the form of thick irregular sheath, deep invaginations and few fibers showed pale stained degenerated myelin sheath. An apparent increase in endoneurium between the nerve fibers was also noticed (Figs. 12 &13).

Electron microscopic examination showed some myelinated nerve fibers with separated myelin lamellae, excessive in folding and few nerve fibers showed myelin loops formation. Myelinating Schwann cells appeared with elongated compressed nuclei (Figs. 14 &15).

Group III (Recovery group):

Light microscopic examination of Hx. & E. stained sections of group III showed nearly regular sciatic nerve structure in the form of regular epineurium, detectable perineurium between the nerve fasciculi and most of the nerve fibers were closely packed. However, some dark Schwann cells nuclei were still noticed, and few areas were

still showing separation of myelinated nerve fibers with loss of some central axons (Figs.16 &17).

Examination of the GFAP immune-histochemical stained sections revealed an apparent mild increase in GFAP positive cells (Fig.18).

Semithin sections stained by toluidine blue showed that most of myelinated nerve fibers appeared having regular myelin sheath of variable thickness with minimal infolding. However, focal thickening and irregularity of myelin sheath of some myelinated nerve fibers and an apparent mild increase in the endoneurium between the nerve fibers were still detected (Figs.19 & 20).

Electron microscopic examination revealed nearly regular myelin sheath with focal lamellar separation of some myelinating nerve fibers. Most of myelinating Schwann cells had large oval nuclei and showed intracellular dark deposits (Figs.21&22).

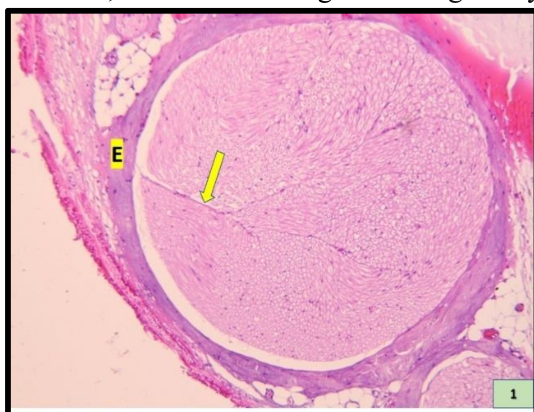

Figure1: A photomicrograph of a transverse section of sciatic nerve of group I, showing densely packed nerve fibers, surrounded by regular epineurium (E). Notice, the perineurium septa separating the fasciculi (arrow). (Hx. & E. X100)

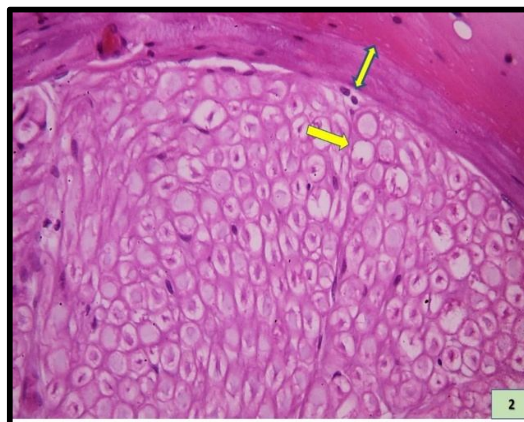

Figure 2: A photomicrograph of a transverse section of sciatic nerve of group I, showing thin perineurium (arrows) surrounding the nerve bundles. Notice the thick vascular epineurium (double headed arrow). (Hx. & E. X400)

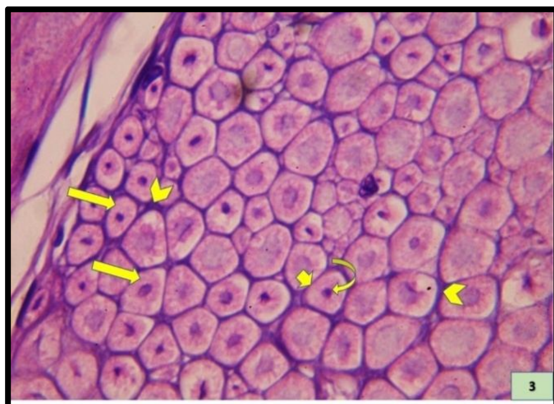

Figure 3: A photomicrograph of a transverse section of sciatic nerve of group I, showing myelinated nerve fibers of variable diameters (long arrows), each nerve fiber formed of a central nerve axon (curved arrow) surrounded by myelin sheath (short arrow). Notice, hardly detected Schwann cells nuclei (arrowhead). (Hx. & E. X1000)

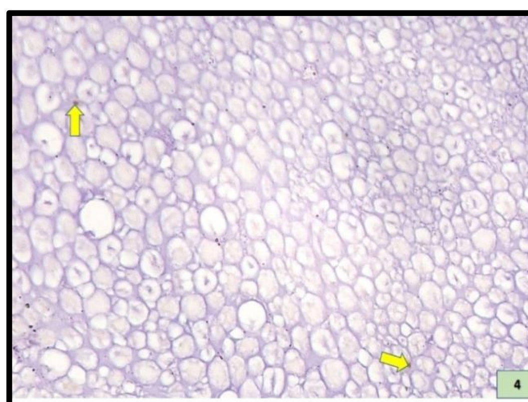

Figure 4: A photomicrograph of a transverse section of sciatic nerve of group I, showing, weak reaction of scattered GFAP positive cells between the nerve fibers. (GFAP X400)

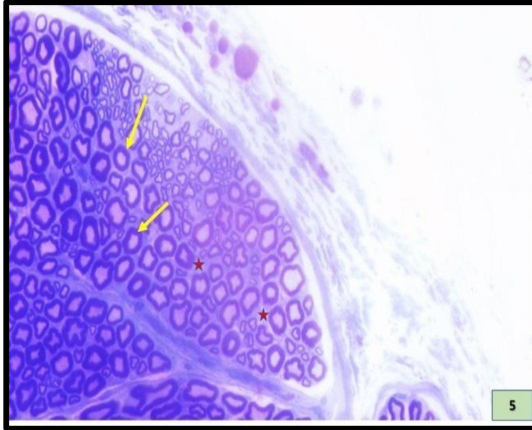

Figure 5: A photomicrograph of a semithin section of the sciatic nerve of group I, showing densely packed myelinated nerve fibers (arrows). Minimal endoneurium between the nerve fibers (stars). (Toluidine blue X 400)

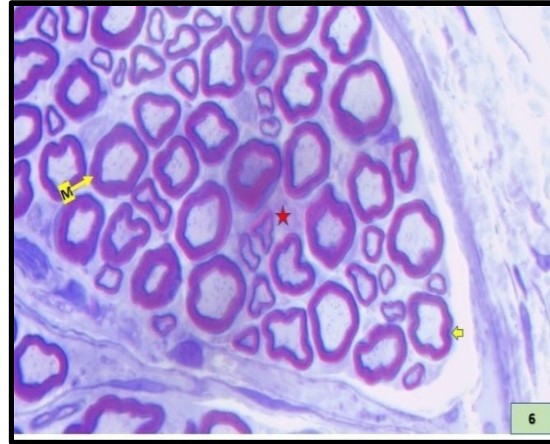

Figure 6: A higher magnification of the previous photomicrograph of a semithin section of group I, showing myelinated nerve fibers with regular myelin (M) and minimal infolding (short arrow) in some nerve fibers. Notice, minimal endoneurium (star) between the nerve fibers. (Toluidine blue X 1000)

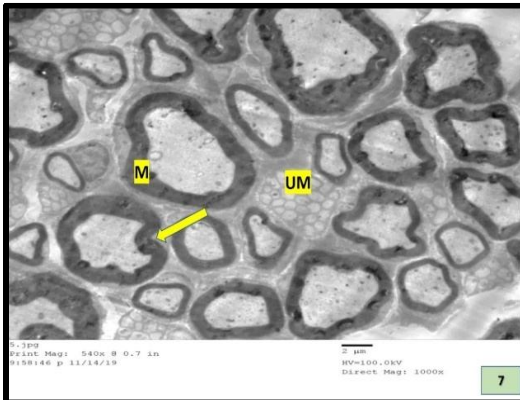

Figure 7: An electron photomicrograph of the sciatic nerve of group I, showing myelinated axons enveloped by uniform thick and compact lamellarmyelin sheath (M) with minimal invaginations (arrow). Notice the presence of unmyelinated axons arranged in group (UM) between the myelinated fibers. (Uranyl acetate & Lead citrate, X1000)

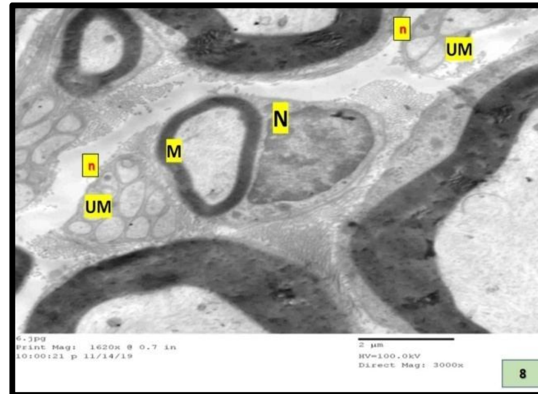

Figure 8: An electron photomicrograph of the sciatic nerve of group I, showing myelinating Schwann cell with large oval nucleus (N) surrounding one myelinated axon (M). Notice the non-myelinating Schwann cell having small pale stained oval nucleus (n) and invaginated by a group of unmyelinated axons (UM). (Uranyl acetate & Lead citrate, X3000)

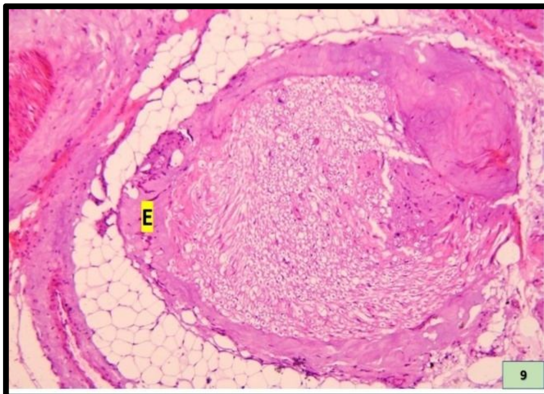

Figure9: A photomicrograph of a transverse section of sciatic nerve of group II, showing thick irregular epineurium (E) and hardly detected perineurium. (Hx. & E. X100)

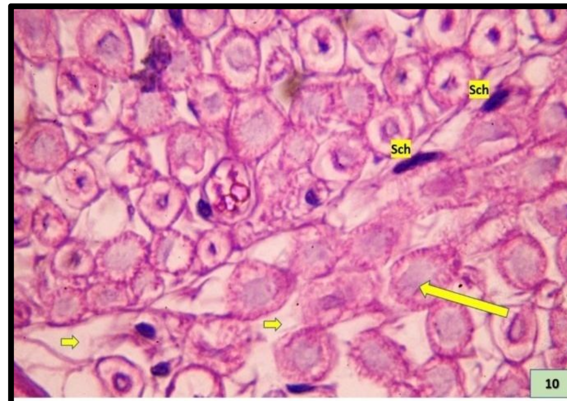

Figure 10: A photomicrograph of a transverse section of sciatic nerve of group II, showing, wide separation (short arrows) of the myelinated nerve fibers with loss of some central axons (long arrow). Notice, the dark elongated Schwann cells nuclei (Sch).
(Hx. & E. X1000)

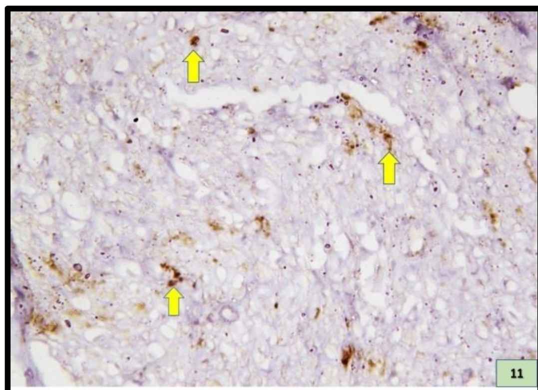

Figure11: A photomicrograph of a transverse section of sciatic nerve of group II, showing, an apparent moderate increase in GFAP positive cells (arrows). (GFAP X400)

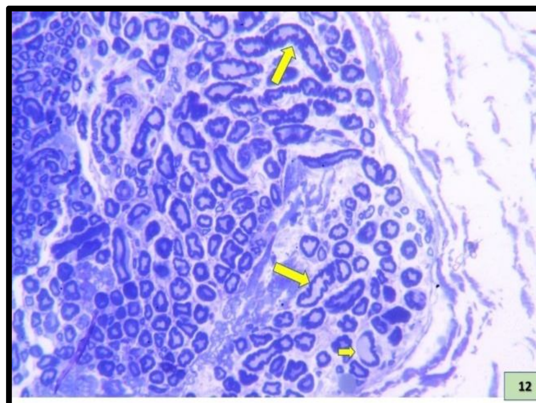

Figure12: A photomicrograph of a semithin section of group II, showing irregular myelinated nerve fibers (long arrows) and few nerve fibers with pale stained degenerated myelin sheath (short arrow).
(Toluidine blue X400)

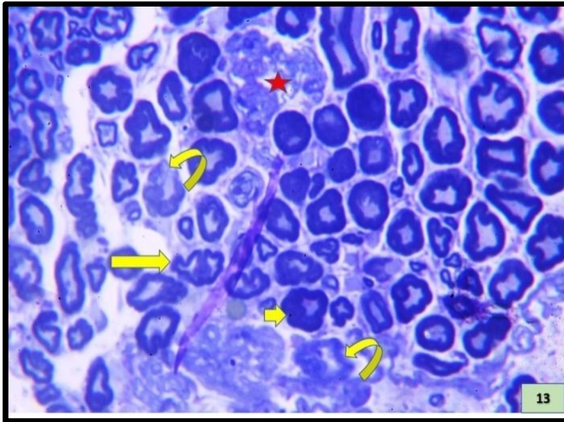

Figure 13: A photomicrograph of a semithin section of group II, showing myelinated nerve fibers with myelin sheath changes, thickened sheath (short arrow), irregular sheath with deep invaginations (long arrow)and few nerve fibers with pale stained degenerated myelin sheath (curved arrow). Notice, an apparent increase in endoneurium (star) (Toluidine blue X1000)

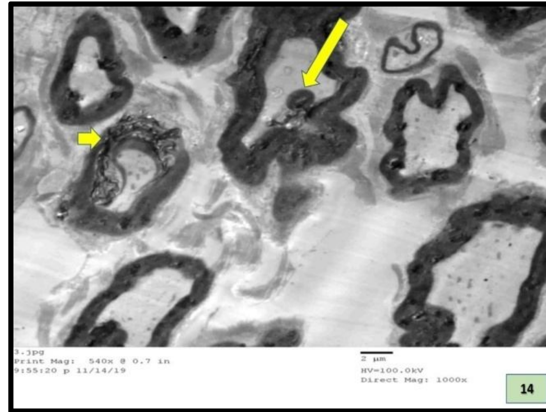

Figure 14: An electron photomicrograph of the sciatic nerve of group II, showing myelinated axon with separated myelin lamellae (short arrow), and another myelinated axon showing excessive in folding of myelin sheath with formation of myelin loop (long arrow).
(Uranyl acetate & Lead citrate, X1000)

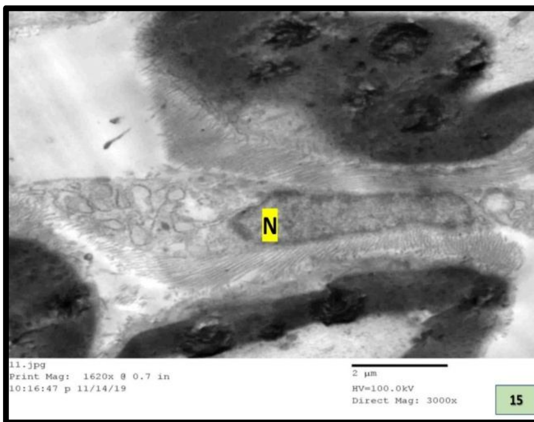

Figure15: An electron photomicrograph of the sciatic nerve of group II, showing myelinating Schwann cell with compressed elongated nucleus (N).
(Uranyl acetate & Lead citrate, X3000)

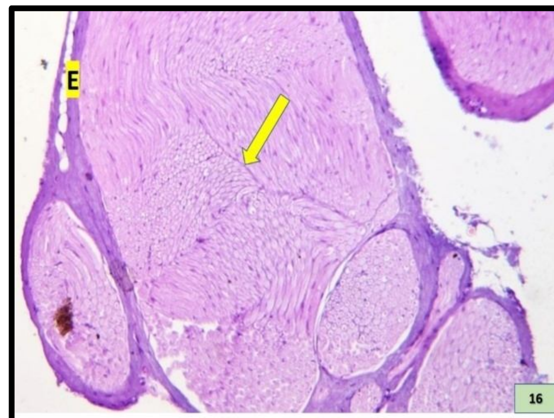

Figure16: A photomicrograph of a transverse section of sciatic nerve of group III, showing nearly regular nerve structure with regular epineurium (E) and detectable perineurium (arrow). (Hx. & E. X100)

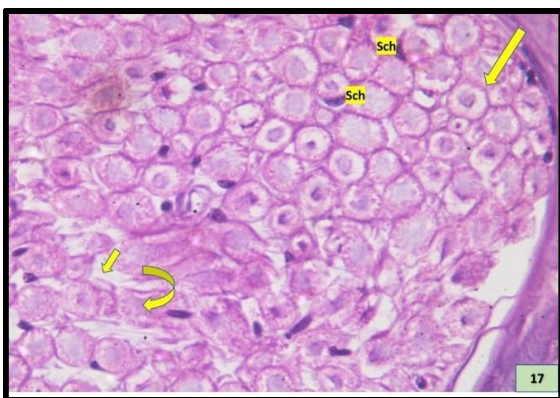

Figure 17: A photomicrograph of a transverse section of sciatic nerve of group III, showing closely packed nerve fibers (long arrow) with dark stained Schwann cells nuclei (Sch). However, areas with separation (short arrow) of myelinated nerve fibers and loss of central axons (curved arrow) still noticed.

(Hx. & E. X1000)

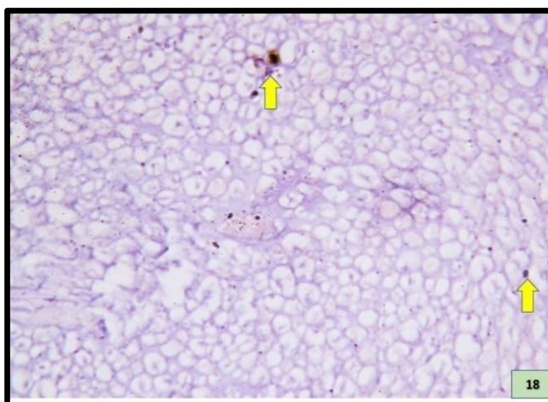

Figure 18: A photomicrograph of a transverse section of sciatic nerve of group III, showing an apparent mild increase in GFAP positive cells (arrows). (GFAP X400)

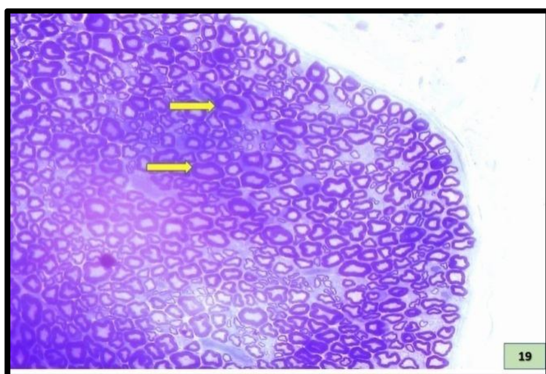

Figure 19: A photomicrograph of a semithin section of group III, showing regularly myelinated nerve fibers (arrows).

(Toluidine blue X400)

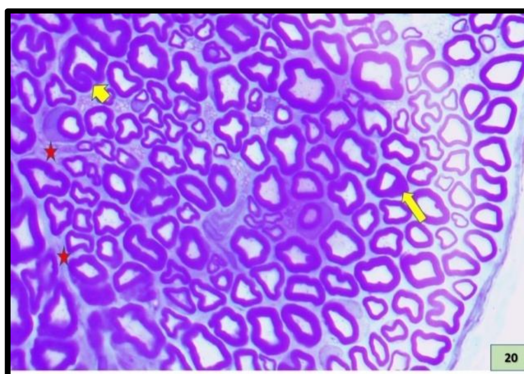

Figure 20:A photomicrograph of a semithin section of group III, showing some myelinated nerve fibers with irregular myelin sheath (short arrow) and others with focal thickening of myelin sheath(long arrow). Notice, an apparent mild increase in endoneurium (stars).

(Toluidine blue X 1000)

Figure 21: An electron photomicrograph of the sciatic nerve of group III, showing some myelinated axons with focal myelin lamellar separation (arrows).
(Uranyl acetate & Lead citrate, X1000)

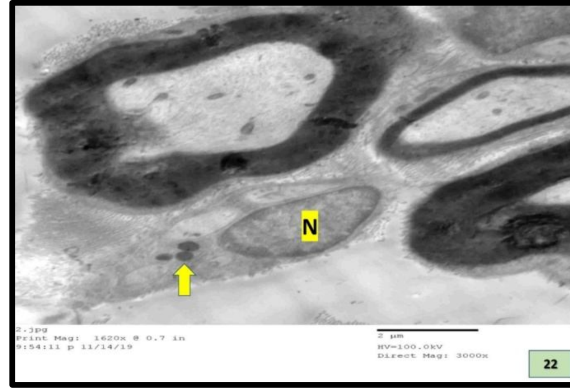

Figure 22: An electron photomicrograph of the sciatic nerve of group III, showing Schwann cell with large oval nucleus (N). Notice the intracellular dark deposits (arrow).
(Uranyl acetate & Lead citrate, X3000)

Morphometrical results:

Morphometric studies were used for measuring the mean thickness of myelin sheath of the myelinated nerve fibers of the sciatic nerve of the three groups. Statistical analysis revealed highly significant increase in group II as compared with group I with P-value < 0.001. Similarly, a highly significant increase in group II as compared with group III with P-value < 0.001 and a significant increase in group III as compared with group I with P-value < 0.05 have been also found (Table 1 and column chart 1).

Comparing the mean thickness of myelin sheath of the sciatic nerve in toluidine blue stained sections, between the three groups showing P-value either, significant (*) or highly significant (**).

<table>
<tr><td colspan="2"></td><td>Group I
(Control)</td><td>Group II
(Aspartame)</td><td>Group III
(Recovery)</td></tr>
<tr><td colspan="2">Myelin sheath thickness
(mean ± standard deviation)</td><td>2.3± 1.0</td><td>8.3± 1.0</td><td>3.7± 0.4</td></tr>
<tr><td rowspan="3">T test</td><td>Between Group I & II</td><td colspan="3">P= 0.0003**</td></tr>
<tr><td>Between Group II & III</td><td colspan="3">P = 0.0003**</td></tr>
<tr><td>Between Group I & III</td><td colspan="3">P= 0.002*</td></tr>
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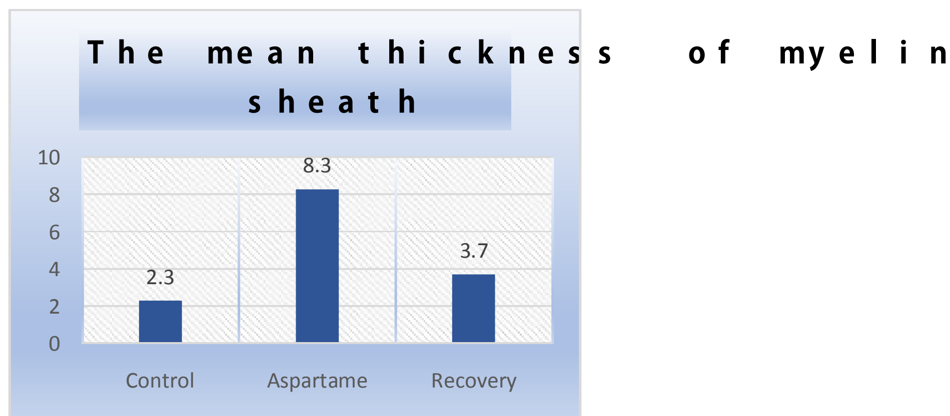

Demonstrating the morphometric comparison between the three groups as regards; the mean thickness of myelin sheath.

DISCUSSION:

Aspartame has been linked to many neurological defects such as headache, cognitive impairments, anxiety, sleep impairment, depression, and impairment in learning and memory(5&28).

The results obtained from the present work revealed that long-term daily aspartame consumption in group II resulted in many histopathological changes of rats' sciatic nerves which were detected mainly in the myelinated nerve fibers and in Schwann cells, Hx. & E. stained sections showed irregular sciatic nerve structure in the form of wide separation of myelinated nerve fibers, degeneration of some central axons, thick irregular epineurium and hardly detected perineurium. The anatomical structure and the molecular components of the physical barriers such as epineurium, perineurium and endoneurium of the peripheral nerves are often influenced by peripheral nerve injury that induced by the inflammatory reactions of many pathological conditions(29). Semithin sections stained with toluidine blue showed thick irregular myelin sheath which was confirmed by the morphometric analysis of the mean thickness of myelin sheath of the myelinated nerve fibers that revealed highly significant increase in group II as compared with the control group. This increase in thickness of myelin sheath was most probably attributed to the splitting of the myelin sheath lamellae(30). In addition, deep invaginations of myelin sheath, degenerated myelin sheath and apparent increase in endoneurium between the nerve fibers were also detected in the present study. Okasha(2) stated that long term aspartame consumption in rats caused degenerative changes in the myelin sheaths.

Moreover, ultra structural examination of group II Sciatic nerves showed myelinated nerve fibers with separated myelin lamellae and excessive in folding with myelin loops formation. Myelin loops have been described normally as a step-in myelin recycling. Increased myelin loops formation is known to be associated with redundant myelin as an early response of myelin sheath to axonal atrophy(31&32). El-Derieny et al.(30) attributed the increase in myelin sheath invaginations and folding to the changes in myelin basic proteins secondary to toxic insult.

Several mechanisms were believed to be involved in the neurotoxic effects of aspartame and they were mostly related to its toxic metabolic products as aspartame is metabolized in the body to phenylalanine, methanol and aspartate(33), methanol is

further metabolized into formaldehyde and formic acid[34]. Aspartate is considered a neurotoxin as it is a substrate for glutamate[5]. Glutamate and aspartate are important neurotransmitters that are normally present in central nervous system but above a certain critical level, they become excitotoxins[2&35]. Parthasarathy et al.[36] and Saito et al.[37] stated that, significant increase in the plasma level of methanol and formaldehyde after even small dose of aspartame was detected and it was associated with free radicals' formation, these free radicals capable of damaging the cellular proteins and the DNA. Ying et al.[38] reported that, the oxidative stress by the free radicals may impair the axonal membrane, leading to demyelination. Furthermore, previous studies added that the increase in the production of free radicals may lead to cell membrane damage by lipid peroxidation or by activating calcium channels leading to increase calcium entry that stimulate the neurons and fire impulses repetitively till cell death[2,39&40].

Regarding, Schwann cells nuclei by Hx. & E. stained sections they appeared dark elongated and by ultra structural examination, Schwann cells had elongated compressed nuclei. Myelinating Schwann cells support the axons and release growth factors to nourish and myelinate the large axons[41-43]. Demyelination can occur following a direct toxic insult to the Schwann cells or its myelin sheath or as a response to axonal degeneration[32].

In addition, immunohistochemical staining for GFAP revealed an apparent marked increase in GFAP positive cells. GFAP is an intermediate filament protein present in the astrocytes of the central nervous system and in the non-myelinating Schwann cells in the peripheral nervous system. Myelin-forming Schwann cells don't express GFAP except if separated from their contact myelinated axons[25&44]. A previous study on human GFAP expression in sciatic nerves revealed that increase GFAP expression was an indicator of nerve pathology [45,46]. This increase in the non-myelinating Schwann cells could be attributed to the ability of the non-myelinating Schwann to respond to nerve injury. After nerve injury, the myelinating Schwann cells degrade their myelin and become non-myelinating cells to regain their developmental potential, including their ability to proliferate, to produce growth factors and to myelinate axons. If they receive the suitable neuronal signals, they eventually promote nerve regeneration[25,44&47].

In the present study incomplete histological improvement was detected in group III rats' sciatic nerves after four weeks of aspartame consumption stoppage. Examination of Hx. & E. stained sections showed nearly regular sciatic nerve structure in the form of regular epineurium, apparent perineurium between the fasciculi and most of the nerve fibers were closely packed. However, areas of separation between the myelinated nerve fibers with loss of their central axons were still detected. By semithin sections stained with toluidine blue, most of myelinated nerve fibers appeared having regular myelin sheath with variable thickness and minimal in folding. However, focal thickening and irregularity of myelin sheath of some myelinated nerve fibers and apparent mild increase in the endoneurium between the nerve fibers were still detected. The morphometric analysis of the mean thickness of myelin sheath revealed a highly significant decrease as compared with group II and a significant statistical increase as compared with the control group. Omar[4] reported that cessation of aspartame administration for four weeks was not enough to obtain a normal histological structure of frontal cortex after chronic aspartame consumption in rats. Furthermore, Ultrastructural examination of group III sciatic nerves revealed nearly regular myelin sheath with the presence of some nerve

fibers that showed focal lamellar separation. This was in agreement with Okasha[2] who reported that ultrastructural examination of the Sciatic nerves of the recovery group that left four weeks after aspartame consumption for long duration showed incomplete recovery.

Regarding, Schwann cells by Hx. & E. stained sections, they were still showing dark nuclei. In addition, Examination of GFAP immunohistochemical stained sections revealed an apparent mild increase in GFAP positive cells. Ultrastructural examination revealed that most of the myelinating Schwann cells had large oval nuclei and showed intracellular dark deposits. Schwann cells were thought to be involved in eliminating myelin debris[19]. Intracellular dark deposits were thought to be the remnants of the phagocytic activity of the myelinating Schwann cells after removing the degenerated myelin sheath [48].

Conclusion:

The results supported the neurotoxic effect of aspartame on rats' sciatic nerves when consumed regularly for a long period and proved that the spontaneous recovery wasn't complete after stoppage administration for four weeks. Further studies by using longer recovery durations are recommended for evaluation of the possibility of complete recovery.

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التأثير العصبي السمى للأسبارتيم على العصب الوركي لذكرالجرذ الابيض البالغ وإمكانية الشفاء التلقائي : دراسة بالمجهر الضوئى والالكترونى

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المقدمة: يعتبر الأسبارتام من أكثر أنواع التحلية الاصطناعية استخدامًا. يتم استخدامه في كثيرمن المنتجات الغذائية والمشروبات المتنوعه. ومع ذلك ، فإن استهلاك الأسبارتام كان مثيرا للجدل حيث ذكرت العديد من الدراسات وجود علاقة بين استخدامه والعديد من الأمراض الخطيرة.

الهدف من العمل: كان الهدف من هذه الدراسة هو تقييم التأثير السمي العصبي المحتمل لاستهلاك الأسبارتام لمدة طويلة على العصب الوركي للجرذان البيضاء الذكور البالغين وإمكانية الشفاء التلقائي بعد توقف تناوله.

المواد والأساليب المستخدمة: تم استخدام ثلاثين ذكرًا من الجرذان البيضاء في هذه الدراسة ، الذين تتراوح أعمارهم بين ٦-٨ أشهر ، ويزنون من ١٨٠الى ٢٠٠ جم.تم تقسيم الجرذان عشوائياً إلى ثلاث مجموعات: **المجموعة الأولى**: عشرة جرذان لم تتلق أي شيء سوى الغذاء والماء ، **المجموعة الثانية**: عشرة جرذان تلقت الأسبارتام (٢٥٠ ملغم / كجم) يوميا لمدة ١٢ أسبوعًا **والمجموعة الثالثة**: عشرة جرذان تلقت أسبارتام كما في المجموعة الثانية ثم تركت لمدة أربعة أسابيع للتعافي.

النتائج: كشف العمل الحالي أن استخدام الاسبرتام ادى الى تغيرات نسيجية في الأعصاب الوركية للجرذان في شكل بنية عصبية غير منتظمة مع انفصال الألياف العصبية المغطاة بالمايلاين عن بعضهامع ظهور قتامةو استطالة فىخلايا شوان ،وقداظهرالفحص بالمجهر الالكترونىانفصالطبقات المايلين والتغلغل المفرط مع تكوين حلقات من المايلين. أظهرت المجموعة الثالثة تحسنًا نسبيًا عن المجموعة الثانيةومع ذلك لم تصل لتركيب المجموعة الضابطة كليا.

الخلاصة: دعمت النتائج التأثير العصبي السمي للأسبارتام على العصب الوركي للجرذان عند تناوله بانتظام لفترة طويلة وأثبتت أن التحسن التلقائي كان جزئيا.