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Original Article

Histological and Immunohistochemical Changes in the Hippocampus of the Adult Male Albino Rats Treated with Amethopterin and the Possible Protective Role of Moringa Leaves Extract

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ABSTRACT

Background: Amethopterin (AMP) is a chemotherapeutic drug used to treat many types of cancer like lung and breast cancers.

Aim of work: This study was done to detect the ameliorating effects of Moringa leaves extract (MLE) against AMP induced hippocampus damage.

Material and Methods: Fifty adult male albino rats were used. They were divided into equal five groups. The 1st group provided with water and food ad libitum. The 2nd group received MLE orally (300 mg/kg b w) twice a week. The 3rd group was injected intraperitoneal with AMP (0.5mg/kg b w) twice a week for four weeks. The 4th group was administrated AMP and MLE at the same timeline. The 5th group was given MLE after four weeks of AMP treatment. By the end of the experiment, the rats were anaesthetized; their brains were dissected and processed for histological and immunohistochemical examination.

Results: Histological examination of the brain exhibited the normal structure of the hippocampus in the 1st and 2nd groups. Hippocampus of the 3rd group showed severe damaged neurons, degeneration of pyramidal cells and vacuolated astrocytes. On the other hand, hippocampus of the 4th group revealed a moderate degree of tissue changes while a good degree of improvement with more or less normal neuronal structure was seen in the 5th group. The intensity of expression of apoptotic protein P53 on hippocampus sections of the 3rd group was strongly positive when compared to the control group. A moderate-strong positive expression was detected in the 4th group while mild-moderate positive expressions in the 5th group.

Conclusion: The present study confirms the possible protective role of MLE against the degenerative changes caused by amethopetrin.

Key Words: Amethopterin, hippocampus, moringa leaves extract, rat

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INTRODUCTION

Chemotherapy is used to treat many types of cancer to stop the growth and eliminate cancer cells. It induces neurotoxicity (*Amptoulach & Tsavaris, 2011; Fahmy et al., 2013 and Gulec et al., 2013*). AMP is an anti-metabolite, anti-neoplastic and anti-folate drug. It has anti-inflammatory properties. It is used to treat certain types of cancer (lung and breast cancers) and autoimmune diseases (rheumatoid arthritis and psoriasis) (*Tousson et al., 2014, 2016*). It enters the cells to disrupt their proliferation and mechanism through inhibition of folic acid reductase enzyme. Folate is essential for biosynthesis of DNA and RNA, so synthesis will be inhibited by amethopterin (*Wielinga et al., 2005*). Also, it causes injury of many organs through oxidative stress mechanism (*Iyyaswamy & Rathinasamy, 2012 and Tousson et al., 2016*).

Moringa Oleifera an edible tree that grows widely in the tropics and subtropics of Asia and Africa. Almost all parts of the plant have been utilized in traditional medicine properties (*Anwar et al., 2007; Mahajanan & Mehta, 2008*). Moringa plant plays a vital role as anti-inflammatory and antioxidant natural compounds (*Shaila et al, 2010 and Minaiyan et al., 2014*). Moringa leaves extract is used as a local treatment in skin inflammation caused by insect bites, fungal or bacterial infections (*Misra et al., 2014*).

P53 tumour suppressor gene is a short-lived protein. It is the most widely mutated gene in oncogenesis. It regulates the transcription rate of several genes that regulate genomic stability, cell-cycle and apoptosis (*Tsurusawa et al., 1997; Collavin et al., 2010; Tousson et al., 2011, 2014, 2016*).

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MATERIAL AND METHODS

Animals

50 adult male albino rats (180 ±10g each) were purchased from the Animal House. The rats were maintained at 21–23 ◦C under normal light–dark cycle. They were assigned randomly to five equal groups (10 animals each) as following:

- Group 1 (Control Group): The rats were provided with water and food ad libitum.
- Group 2 (MLE Group): They received MLE orally 300 mg/kg b w/twice a week (*Rathi et al., 2006*).
- Group 3 (AMP-treated Group): Rats were subjected to acclimatization period of one week before the experiment. They were injected AMP in the peritoneum (0.5 mg /kg b w/twice a week) for 4 weeks according to *Tousson et al. (2016)*.
- Group 4 (AMP and MLE-treated animals): The rats were injected AMP as the 3rd group and received MLE as the 2nd group at the same timeline.
- Group 5: The rats were injected AMP as the 3rd group for 4 weeks then treated with MLE as the 2nd group for another 4 weeks.

After the exposure period for all groups, animals were anaesthetized, dissected and the brain was excised and processed for histological and immunohistochemical studies.

I- Histological study:

The specimens from the brain were fixed in buffered neutral formalin, post-fixed, dehydrated, cleared and stained with Hx.&E (Bancroft & Gamble 2008).

II- Immunohistochemical study (P53 immunoreactivity):

Affixed paraffin slides were waxed in xylene, hydrated, treated with 0.3% hydrogen peroxide for endogenous blocking of peroxidase and non-specific binding sites for antibodies. Slides were subjected to heating for 20 minutes in a microwave at pH=6.0. Dilutions (1:80) of primary antibodies (Dako, Glastrup, Denmark) were performed at the room temperature for two hours.

RESULTS

I- Histological examination

Histological examination of the brain exhibited the normal architecture of the hippocampus in the control and MLE groups. The hippocampus is a curved shape like Cornu Ammonis (CA) or sea-horse. It consisted of four regions CA1, CA2, CA3 and CA4. It was formed of pyramidal cells and astrocytes. CA4 received afferent fibres while all fibres exit from CA1 (Figs. 1, 2 & 3).

In contrast, light microscopic examination of the hippocampus sections of amethopterin group showed numerous histopathological changes including a large number of damaged neurons, degenerated pyramidal cells and vacuolated neurocytes. Nuclei of the cells were shrunken, pyknotic and hyperchromatic. Also; many of damaged apoptotic astrocytes were in the form of pyknotic, shrunken and vacuolated neurons (Figs. 4 & 5).

On the other hand, hippocampus of the 4th group showed a moderate tissue changes as mild diffuse vacuolar degeneration and a few apoptotic astrocytes in the form of pyknotic neurons (Fig. 6). In the 5th group, a good degree of improvement with more or less normal neuronal structure was observed. However, a mild atrophy of the neuron was shown (Fig. 7).

II- P53 immunoreactivity in rat Hippocampus:

The apoptotic cells were detected by the tumor suppressor gene P53. Immunohistochemical observation for P53 protein was undetectable in the control (Fig. 8) and MLE (Fig. 9) groups. On the other hand, immunohistochemical study of the AMP-treated group revealed strong positive reaction for P53 gene (Fig. 10). In the 4th group; a moderate-strong positive expression was seen (Fig. 11) while immunohistochemical study of the 5th group revealed mild-moderate immunoreactivity (Fig. 12). The intensity of expression of P53 gene on hippocampus sections in the treated amethopterin with MLE was less than that of the amethopterin group.

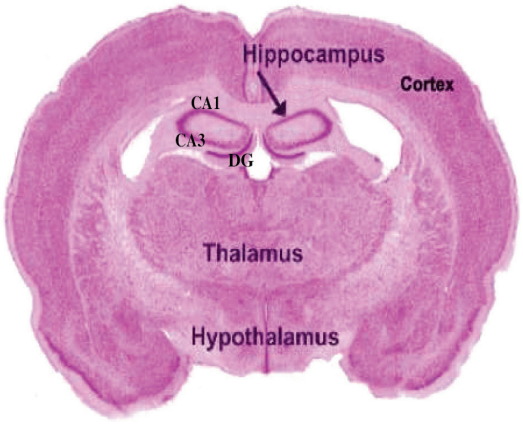

Fig.1: A photomicrograph of coronal section of control rat brain stained with Haematoxylin & Eosin showing cerebral cortex, hippocampus Cornu Ammonis (CA1, CA3), dentate gyrus (DG), thalamus and hypothalamus. (Hx & E; Scale bar, 1 mm).

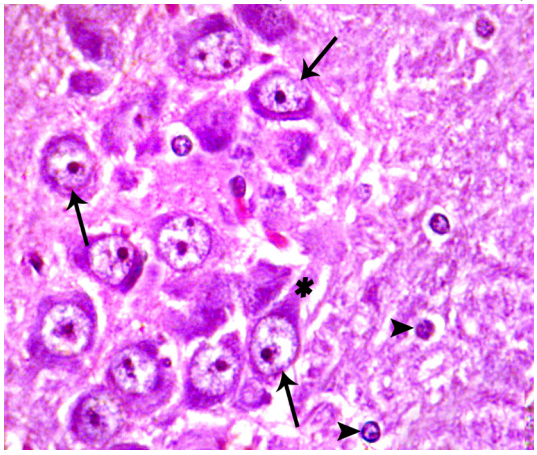

Fig. 2: A photomicrograph of rat hippocampus coronal section in the control group (1st group) stained with Haematoxylin & Eosin revealed normal structure of pyramidal cells (arrows), astrocytes (arrow heads) and fibers(*). (Hx & E; X 400).

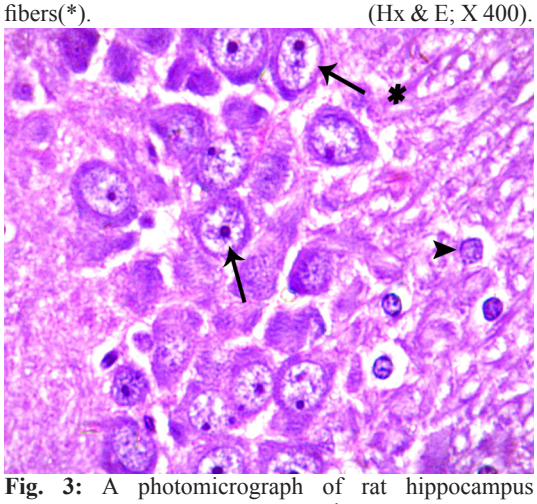

Fig. 3: A photomicrograph of rat hippocampus coronal section in MLE group (2nd group) stained with Haematoxylin & Eosin showing normal structure of pyramidal cells (arrows), astrocytes (arrow head) and fibers(*). (Hx & E; X 400).

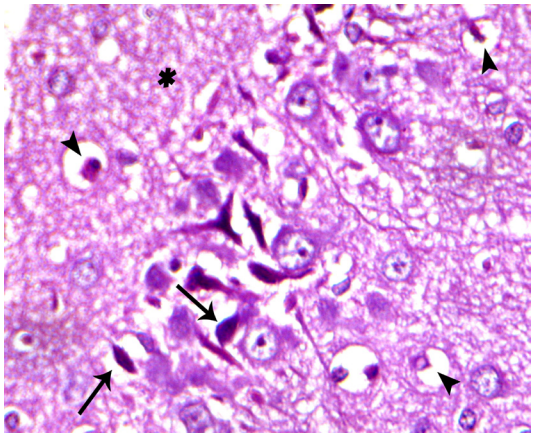

Fig. 4: A photomicrograph of rat hippocampus coronal sections in Amethopterin treated group (3rd group) stained with Haematoxylin & Eosin showed a large number of degenerated and vacuolated pyramidal cells (arrows) and astrocytes (arrow heads). The fibers (*) were also disturbed. (Hx & E; X 400).

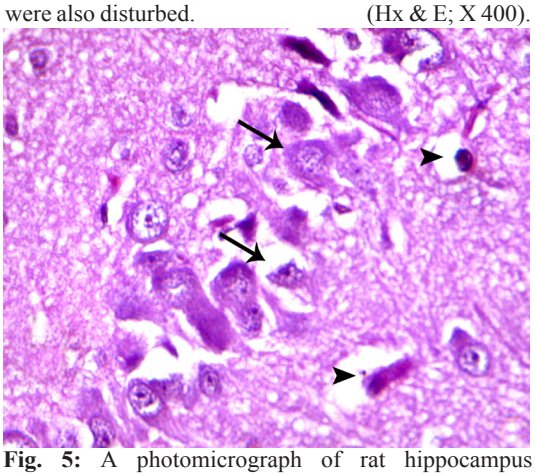

Fig. 5: A photomicrograph of rat hippocampus coronal sections in Amethopterin treated group (3rd group) showing degenerated and vacuolated pyramidal cells (arrows) and astrocytes (arrow heads). (Hx & E; X 400).

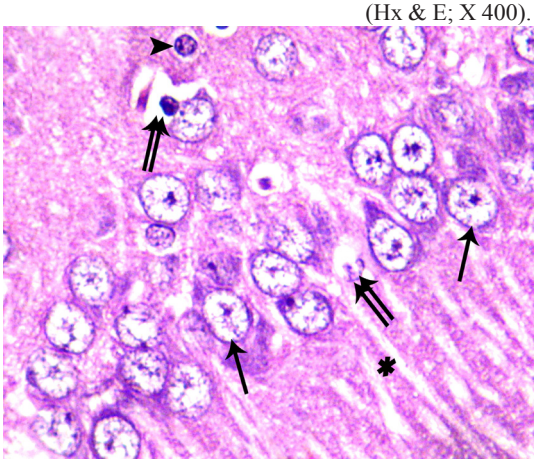

Fig. 6: A photomicrograph of a rat hippocampus coronal section in the co-treated Amethopterin with MLE (4th group) stained with Haematoxylin & Eosin revealed a mild diffuse vacuolar degeneration of pyramidal cells (double arrows). Normal pyramidal cells (arrows), fibres (*) and astrocytes (arrow head) were observed. (Hx & E; X 400).

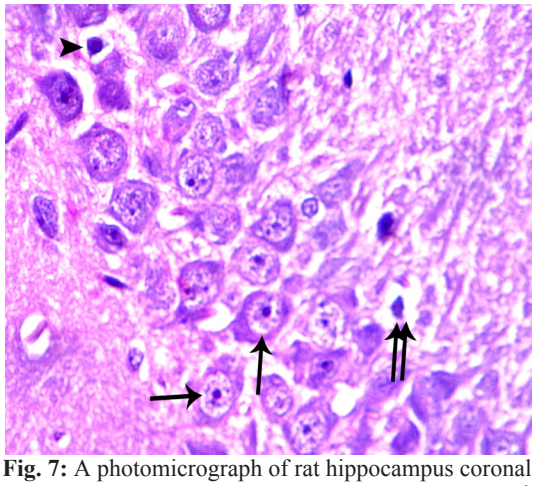

Fig. 7: A photomicrograph of rat hippocampus coronal section in post treated Amethopterin with MLE (5th group) stained with Haematoxylin & Eosin showed normal pyramidal cells (arrows) and astrocytes (arrow head). A mild atrophy of pyramidal cells (double arrow) was also observed. (Hx & E; X 400).

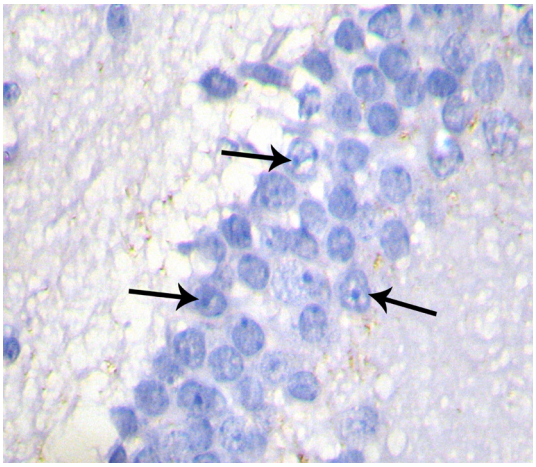

Fig. 8: A photomicrograph of rat hippocampus coronal section in control group (1st group) stained with P53 immunoreactivity showed undetectable reaction (arrow) for P53 gene. (P53; X 400).

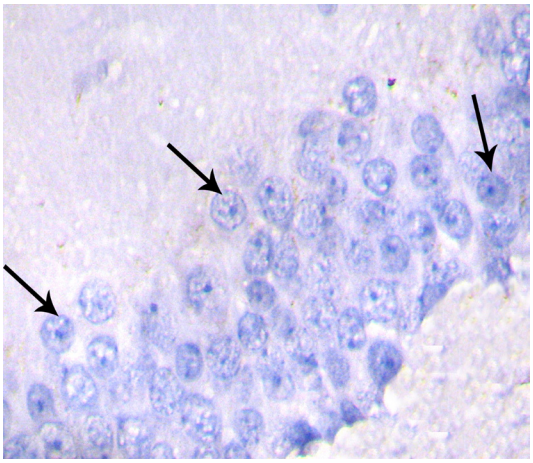

Fig. 9: A photomicrograph of a rat hippocampus coronal section in MLE group (2nd group) stained with P53 immunoreactivity showed undetectable reaction (arrows) for P53 gene. (P53; X 400).

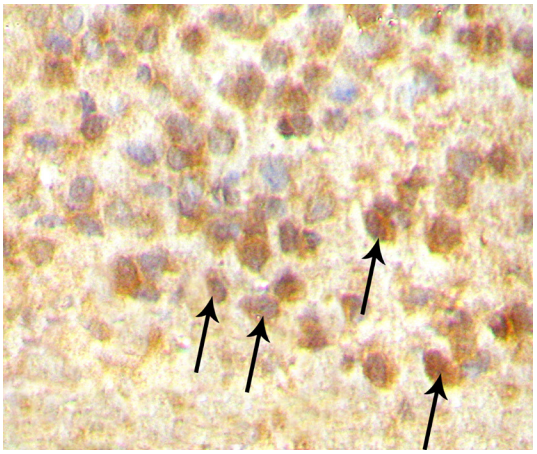

Fig. 10: A photomicrographs of rat hippocampus coronal sections in Amethopterin treated group (3rd group) stained with P53 immunoreactivity showed strong positive expressions of P53 gene (arrows). (P53; X 400).

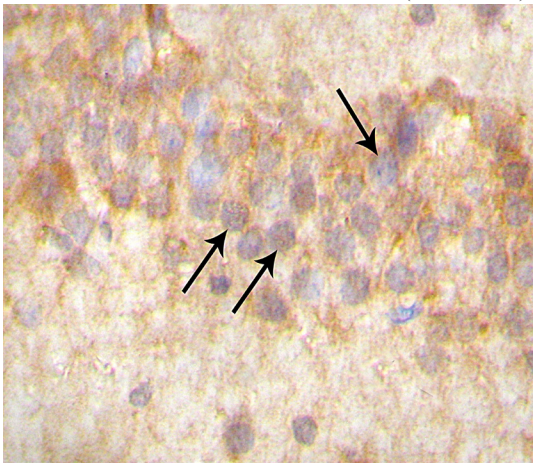

Fig. 11: A photomicrograph of rat hippocampus coronal section in co-treated Amethopterin with MLE (4th group) stained with P53 immunoreactivity showed moderate- strong positive expressions of P53 gene (arrows). (P53; X 400).

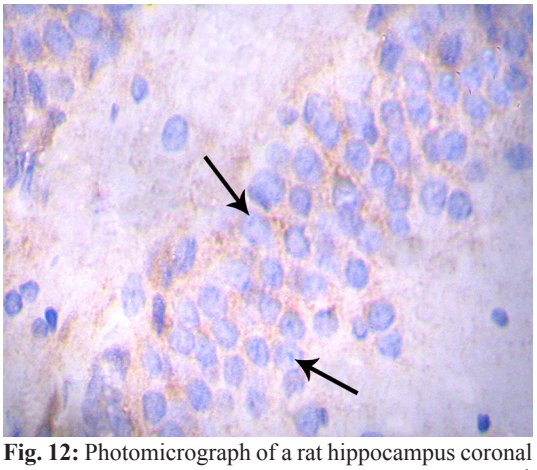

Fig. 12: Photomicrograph of a rat hippocampus coronal section in the post treated Amethopterin with MLE (5th group) stained with P53 immunoreactivity showed mild- moderate positive expressions of P53 gene (arrows). (P53; X 400).

DISCUSSION

The hippocampus is a part of limbic system. It is responsible for memory and learning. The blood-brain barrier protects the brain from some drugs but many chemotherapeutic drugs can affect its function by direct or indirect methods (*Aggleton, 2012*).

The histological study showed that AMP induces numerous histopathological changes in the hippocampus as degenerated and vacuolated astrocytes and pyramidal cells. The same results were reported by *Al Moundhri et al. (2013)* after administration of cisplatin. Treatment of the breast cancer in the mouse model by methotrexate led to hippocampal dysfunction and changes *(Yang et al., 2012)*. The results of *Winocur et al. (2006)* revealed that combination treatment of methotrexate and 5-FU lead to damage of mice testes. *Tousson et al. (2016)* reported that; AMP induced oxidative stress, injury and apoptosis in cardiac muscles. The previous authors also reported that the cardiac damage can be protected by administration of L-carnitine. *The research of Reiriz et al. (2006)* also revealed transient acute memory impairment in the mice after cyclophosphamide treatment with a single dose. These previous studies agreed with the results of this study. On the hand, *Yoshikawa et al. (2005)* reported that no changes in the hippocampal volume after treatment of Japanese breast cancer by chemotherapy. *Kim et al. (2010)* presumed that the irreversible cell damage after cisplatin administration due to oxidative stress. Amethopterin prevents conversion of folic acid to folinic acid (active form) through binding dihydro folic reductase enzyme leading to neuronal damage. The active form of the folic acid is important to synthesis of certain amino acids and nucleic acids *(Tousson et al., 2016)*.

The present study showed that an administration of MLE enhanced the hippocampal changes induced by amethopetrin. The same findings were reported by *Anwar et al., (2007); Mahajan & Mehta (2008); Shaila et al. (2010); Minaiyan et al. (2014) and Misra et al. (2014)*.

P53 tumor suppressor protein regulates the transcription rate of several genes involved apoptosis *(Tousson et al., 2011, 2014, 2016)*. The immunohistochemical results exhibited a significant increase of P53 (apoptotic protein) in amethopetrin-treated group. The increasing of immunoreactivity in amethopetrin-treated group revealed the possibility of apoptosis. On the other hand, the expression of P53 decreased in groups received MLE after injection of amethopetrin. Similar results for Bcl-2 and P53 were observed in lung and cardiac muscles after AMP administration *(Tousson et al., 2014, 2016)*.

CONCLUSION

The present study confirms the possible role of MLE in protecting cells against the degenerative changes and apoptosis caused by amethopetrin without affecting cell proliferation.

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تغييرات هستولوجية وهستوكيميائية مناعية في حصين ذكورالجرذان البيضاء البالغة المعالجة بأمي توبيرن والدور الوقائي المحتمل لمستخلص أوراق المورينجا

يوسف حسين

قسم التشريح الآدمى وعلم الاجنة ، كلية الطب ، جامعة الزقازيق

ملخص البحث

مقدمة : أمي توبيرن دواء العلاج الكيميائي يستخدم لعلاج أنواع عديدة من السرطان مثل سرطانات الرئة والثدى.

الهدف من البحث : أجريت هذه الدراسة للكشف عن الآثار التحسينية لمستخلص أوراق المورينجا ضد الضرر الناجم في الحصين من أمي توبيرن.

مواد وطرق البحث : تم استخدام خمسون من ذكور الجزذان البيضاء البالغة. تم تقسيمهم إلى خمس مجموعات متساوية. المجموعة الأولي تم تزويدها بالماء والغذاء. المجموعة الثانية تم اعطائها مستخلص أوراق المورينجا 300 ملجم / كجم من وزن الجسم مرتين في الأسبوع عن طريق الفم. المجموعة الثالثة تم حقنها بأمي توبيرن في التجويف البريتوني 0.5ملجم / كجم من وزن الجسم مرتين في الأسبوع لمدة أربعة أسابيع. المجموعة الرابعة تم اعطائها أمي توبيرن و مستخلص أوراق المورينجا في نفس الفترة الزمنية. المجموعة الخامسة تم اعطائها مستخلص أوراق المورينجا بعد اربع اسابيع من أمي توبيرن. في نهاية التجربة ، تم تخدير الجرذان ثم استخراج المخ وتحضيره للفحص هستولوجيا، وهستوكيميائية مناعية.

النتائج : الفحص النسيجي للدماغ أظهر البنية الطبيعية للحصين في المجموعة الاولي والثانية. ظهرت تغيرات في حصينات المجموعة الثالثة مثل تلف شديد في الخلايا العصبية، تدهور الخلايا الهرمية وظهور تجاويف في الخلايا النجمية. من ناحية أخرى، أظهرت حصينات المجموعة الرابعة درجة معتدلة من التغيرات النسيجية في حين كانت درجة التحسن فى المجموعة الخامسة جيدة في هيكل الخلايا العصبية. كانت كثافة البروتين ب53 (P53)في المجموعة الثالثة إيجابية بقوة مقارنة بالمجموعة الضابطة و كانت كثافة البروتين في المجموعة الرابعة تتراوح من متوسطة الي قوية بينما في المجموعة الخامسة كانت تتراوح من معتدلة الي متوسطة.

الخلاصة : هذه الدراسة تؤكد على الدور الوقائي المحتمل لمستخلص أوراق المورينجا ضد التغيرات التنكسية الناجمة عن أمي توبيرن.