Effect of long-term administration of metanil yellow on the structure of cerebellar cortex of adult male albino rat and the possible protective role of anise oil: A histological and immunohistochemical study

Walaa M. Elwan

Department of Histology, Faculty of Medicine, Tanta University, Tanta, Egypt

ABSTRACT

Introduction: Metanil yellow is used as a colorant in many food processing industries. It has toxic effects on some organs in humans and animals. Anise oil, an essential oil of the flowering plant Pimpinella anisum L., possesses a wide range of pharmacological activities and a proposed role in combating some neurological disorders.

Aim: To study the effect of long term administration of metanil yellow on the structure of cerebellar cortex of adult male Albino rats and to evaluate the possible protective role of anise oil.

Materials and Methods: Forty-five adult male albino rats were divided into four groups; Group I (control), group II further divided into two subgroups (a and b) that received anise oil (0.5ml/kg) orally for four and eight weeks, respectively., Group III also divided into two subgroups (a and b) orally administered metanil yellow (200 mg/kg) for four and eight weeks, respectively. Alike, group IV divided into subgroups (a and b) received both anise oil and metanil yellow at the same dose, route and duration as in groups II and III, respectively. Specimens from the cerebellar cortex were processed for light microscopy. Immunohistochemical study was carried out using antibodies against active caspase-3 and glial fibrillary acidic protein (GFAP).

Results: Specimens from metanil yellow-treated animals showed a high significant decrease in the mean number of Purkinje cells, which appeared shrunken, distorted and surrounded by large vacuolar spaces. Some of their nuclei were shrunken and deeply stained, while others were fragmented. The molecular and granular layers displayed prominent perineuronal spaces. Immunohistochemically, highly significant increases in active caspase-3 and GFAP immunoreactions were observed. In contrast, minimal changes were observed in rats treated concomitantly with metanil yellow and anise oil.

Conclusion: Metanil yellow-induced structural changes in cerebellar cortex of adult rat that could be ameliorated by concomitant treatment with anise oil.

Received: 05 July 2017, Accepted: 11 January 2018

Key Words: Active caspase-3, anise oil, cerebellar cortex, GFAP, metanil yellow.

INTRODUCTION

Synthetic food colors, also called colorants are substances added to food to enhance the aesthetic appeal of food making the food more attractive, and thus increasing the appetite[1–5]. The maximum limit of permitted colors to be added in any food should be 100 mg kg-1 or litre-1 of food; however, excessive amounts of food colors are used in most of cases to give the intended attractive color of the processed food[6].

In Egypt and other developing countries, the uncontrolled use of synthetic food coloring agents has been sharply increased particularly in food used for children nutrition[6]. Therefore, more attention must be focused on the pathophysiological effects of color additives.

Metanil yellow is a water soluble monoazo acid dye, with chemical formula C18H14N3O3SNa. It has wide applications in textiles, cosmetics, plastic laboratories paper printing, leather colour photography and pharmaceutical purposes[5, 6]. Although its use as a colorant is not permitted, it is still used in many food processing industries as a colorant in ice-creams, soft drinks, sweet meat and beverages[7].

Metanil yellow has been documented to be a potent toxic chemical due to its many toxic effects on some organ systems in humans as well as animals[8]. In humans, oral administration of metanil yellow induced toxic methaemoglobinemia[9]. Moreover, colour photography and pharmaceutical purposes[4, 5]. Although its use as a colorant is not permitted, it is still used in many food processing industries as a colorant in ice-creams, soft drinks, sweet meat and beverages[7].
Metanil yellow has been documented to be a potent toxic chemical due to its many toxic effects on some organ systems in humans as well as animals\(^9\). In humans, oral administration of metanil yellow induced toxic methaemoglobinemia\(^9\). Moreover, allergic dermatitis results from its direct contact with the skin\(^9\). A tumor promotion by metanil yellow has been also documented by some authors\(^10\).

Laboratory data revealed that the administration of metanil yellow in the laboratory animals causes many organ toxicities such as testicular lesions\(^12\), alteration of haematopoietic system and lesions in the stomach, intestine, liver and kidney\(^13\). In addition, chronic consumption of metanil yellow and other synthetic colorants caused brain toxicity resulting in imbalance in neurotransmitters\(^14, 16\).

Anise oil is an essential oil of *Pimpinella anisum* L., a flowering plant belonging to the Apiaceae family\(^16\). It has been reported to possess a wide range of pharmacological activities such as antioxidant and anti-inflammatory\(^17\), antibacterial and antiviral\(^18\), antifungal\(^19\), antidiabetic\(^20\), in addition to anticancer effects\(^21\). Moreover, it has been used to promote lactation in nursing mothers\(^22\), and it can also stimulate immunity\(^23\). However, few studies have investigated the effect of this essential oil on neurological disorders\(^24\).

Based on the aforementioned data, this study aimed to investigate the effect of long term administration of metanil yellow on the histological structure of the cerebellar cortex of adult albino rat, using different histological and immunohistochemical techniques.

### MATERIALS AND METHODS

The present study was carried out on 45 adult male albino rats weighing 180-200 grams each. All animals were housed under standard laboratory conditions with free access to food and water throughout the study period. They were acclimatized to the experimental environment at least 2 weeks before starting the study. The experimental procedures were approved by the local Institutional Animal Ethical Committee of Faculty of Medicine, Tanta University, Egypt.

The animals were divided into four groups: Group I (Control): It included 15 rats subdivided into two subgroups; subgroup Ia (5 rats) that received no treatment, and subgroup Ib (10 rats) that was further subdivided into two equal subgroups (Ib1 and 2) and administered 0.5 ml/100 gm body weight of distilled water orally once daily for 4 and 8 weeks, respectively.

Group II (Anise oil -treated): 10 rats subdivided into two equal subgroups Iia and b that were given 0.5ml/kg body weight of anise oil orally via a gastric tube once daily for 4 and 8 weeks, respectively according to Abdul-Hamid and Gallalay (25). Anise oil was purchased from MOBACO Company, Cairo, Egypt.

Group III (Metanil yellow–treated): It included 10 rats subdivided into two equal subgroups IIIa and b that received metanil yellow (200 mg/kg) dissolved in distilled water orally via a gastric tube once daily for 4 and 8 weeks, respectively according to that reported by Al-Malki and Al-Sayed\(^26\). The concentration of metanil yellow was prepared in such a way that each rat received 0.5 ml of metanil yellow/100 gm body weight. The chosen dose was lower than 1/20 of LD50\(^27\). Metanil yellow was purchased from Sigma Chemicals (Sigma, St Louis, MO) (cat# 202029) in the form of 100 gm powder in a glass bottle.

Group IV (Anise oil and metanil yellow-treated): It included 10 rats subdivided into two equal subgroups Iva and b that were concomitantly given both anise oil and metanil yellow at the same doses, routes and duration as in groups II and III, respectively.

At the end of the experiment, all rats were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg body weight)\(^28\). The scalp had been reflected, then the sagittal suture was traced with dissecting blade to obtain the cerebellum to be processed for histological and immunohistochemical study.

For light microscopy; specimens were immersed in 10% neutral-buffered formalin, washed, dehydrated, cleared and embedded in paraffin. Sections of 5µm thickness were stained with haematoxylin and eosin (H&E)\(^29\).

For immunohistochemistry; 5µm thick sections were dewaxed, rehydrated, and washed with phosphate buffered saline (PBS). The sections were then washed in PBS and incubated for 10 min at room temperature with 10% goat serum to block unspecific binding. The sections were then incubated overnight in a humid chamber with the primary antibody at 4°C (rabbit polyclonal anti-rat activated caspase-3 antibody (ab2302; Abcam, Cambridge, Massachusetts, USA), rabbit polyclonal anti-rat glial fibrillary acidic protein (GFAP) (ab116010).Washing in PBS buffer and co-incubation with biotinylated secondary antibody for one hour at room temperature was carried out. Streptavidin peroxidase was then added for 10 minutes and rinsed again three times in PBS. Immunoreactivity was visualized using 3, 3’diaminobenzidine (DAB)-hydrogen peroxide as a chromogen. Sections were counterstained with Mayer’s haematoxylin. The negative control sections were prepared by excluding the primary antibodies (30). Positive controls for active caspase-3 were camptothecin-treated Jurkat cells. Positive controls for GFAP were human brain or astrocytoma tissues. Active caspase-3-immunostained cerebellar sections were considered positive upon expressing clear evident brown nuclear and/or cytoplasmic coloration. GFAP-immunostained cerebellar sections were considered positive upon expressing clear evident brown cytoplasmic coloration.

#### Morphometric study

The images were obtained using a Leica microscope (DM3000, Leica, Germany) coupled to a CCD-camera.
Histological results:

Group I (control): Control rats showed the cerebellar cortex formed of three layers; molecular layer, Purkinje cell layer and granular cell layer (Fig. 1). The molecular layer contained numerous nerve fibers together with few superficial small stellate cells and deep basket cells. The Purkinje cell layer was the layer bordering the molecular and granular cell layers, and it consisted of flask shaped cells having rounded vesicular nuclei with prominent nucleoli. The granular cell layer was composed of closely packed small rounded cells with deeply stained nuclei together with non-cellular areas (cerebellar islands or glomerulus) representing the synapses between the axons entering the cerebellum from outside and the dendrites of granular cells (Fig. 2).

Group II (anise oil-treated): It showed the same histological structure as the control group.

Subgroup IIIa (metanil yellow–treated for 4 weeks): Examination of H&E-stained sections showed disorganized Purkinje cell layer and displacement of Purkinje cells downwards in the granular layer and upwards in the molecular layer (Fig. 3). In addition, an apparent reduction in the number of Purkinje cells was noticed and the few seen Purkinje cells appeared irregular and shrunken with darkly stained nuclei and deeply stained cytoplasm. Some astrocytes in the granular and molecular layers exhibited a moderate positive cytoplasmic immunoreaction with GFAP in approximately few cells in the three cortical layers (Fig. 12). Subgroup IIIa revealed weak positive cytoplasmic immunoreaction in few cells within the Purkinje cell layer (Fig. 13). In subgroup IV b, some cells in the Purkinje cell layer exhibited a moderate positive cytoplasmic immunoreaction (Fig. 14).

GFAP immunostaining:

In the control group, sections immunostained with GFAP showed few astrocytes in the granular and occasionally the molecular layers exhibiting a weak positive cytoplasmic immunoreaction (Fig. 15). In subgroup IIIa, many astrocytes in the three cortical layers showed a strong positive cytoplasmic immunoreaction (Fig. 16). In subgroup IIIb, the GFAP positive cells were more numerous in the three cortical layers (Fig. 17). In subgroup IVa, a moderate positive cytoplasmic immunoreaction was detected only in few astrocytes in the granular and molecular layers (Fig. 18). While, in subgroup IV b, some astrocytes in the granular and the molecular layers exhibited a moderate positive cytoplasmic immunoreaction (Fig. 19).
**Morphometric and statistical analysis (Table 1, Histogram 1):**

The mean number of Purkinje cells was highly significantly decreased in subgroups IIIa and b compared to groups I and II. In subgroups IVa and b, it was highly significantly increased compared to subgroups IIIa and b, respectively but, it still showed a high significant difference compared to groups I and II.

The mean number of active caspase-3 immunohistochemical positive cells was highly significantly increased in subgroups IIIa and b compared to groups I and II. In subgroups IVa and b, it was highly significantly increased compared to subgroups IIIa and b, respectively but, it still showed a high significant difference compared to groups I and II.

The mean percentage (%) of GFAP immunohistochemical positive cells was highly significantly increased in subgroup IIIa and b compared to groups I and II. In subgroups IVa and b, it was highly significantly increased compared to subgroups IIIa and b, respectively but, it still showed a high significant difference compared to groups I and II.

**Table 1: Morphometric analysis of the cerebellar cortex specimens of all groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Subgroup IIIa</th>
<th>Subgroup IIIb</th>
<th>Subgroup IVa</th>
<th>Subgroup IVb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of Purkinje cells/mm</td>
<td>25.81±1.83</td>
<td>25.45±2.98</td>
<td>7.43±0.16a,b</td>
<td>5.21±0.73a,b</td>
<td>19.83±1.27a,b,c</td>
<td>15.75±1.65a,b,d</td>
</tr>
<tr>
<td>Mean number of active caspase-3 cells/20mm²</td>
<td>0.41±0.02</td>
<td>0.32±0.01</td>
<td>20.31±2.56a,b</td>
<td>26.59±2.71a,b</td>
<td>9.14±1.05 a,b,c</td>
<td>11.99±1.78 a,b,d</td>
</tr>
<tr>
<td>Mean percentage (%) of GFAP</td>
<td>8.42±1.24</td>
<td>8.31±1.17</td>
<td>34.62±1.82a,b</td>
<td>38.82±1.27a,b</td>
<td>12.36±1.12 a,b,c</td>
<td>15.08±1.17 a,b,d</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation, a: $p<0.001$ is highly significant versus group I, b: $p<0.001$ is highly significant versus group II, c: $p<0.001$ is highly significant versus subgroup IIIa, d: $p<0.001$ is highly significant versus subgroup IIIb

**Histogram 1:** Morphometric analysis on biometric values of the cerebellar cortex specimens of all groups. (A) Mean number of Purkinje cells/mm length of cerebellar lobules. (B) Mean number of active caspase-3 positive cells/20mm². (C) The mean percentage of GFAP.
Fig. 1: A photomicrograph of a section in cerebellar cortex of control group I showing the cerebellar cortex composed of three layers; molecular (M), Purkinje (P) and granular (G) layers (H&E x200).

Fig. 2: A photomicrograph of a section in cerebellar cortex of control group I showing the molecular (M) containing numerous nerve fibers together with few superficial small stellate cells (arrow heads) and deep basket cells (notched arrows). The Purkinje layer (P) consists of flask shaped cells (arrows) having rounded vesicular nuclei with prominent nucleoli. The granular (G) layer is composed of closely packed small rounded cells with deeply stained nuclei (wavy arrows) together with non-cellular cerebellar islands (asterisks) (H&E x400).

Fig. 3: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIa showing disorganized Purkinje layer (P) with displacement of Purkinje cells downwards in the granular layer (G) (asterisks) and upwards in the molecular layer (M) (arrowhead) (H&E x400).

Fig. 4: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIa showing an apparent reduction in the number of Purkinje cells. Shrunken Purkinje cells with deeply stained nuclei and eosinophilic homogenization of their cytoplasm (arrows) are noticed. Unstained haloes (asterisks) are seen surrounding the Purkinje cells. The inset shows an irregular Purkinje cell with ill-defined nucleus and eosinophilic homogenization of cytoplasm (arrow head). (H&E x400, inset x1000).

Fig. 5: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIb showing an apparent marked reduction in the number of Purkinje cells. Purkinje cells are distorted and shrunken (arrows), and surrounded many nuclei of neuroglia (arrowheads). The molecular (M) and granular (G) layers displayed prominent perineuronal spaces (asterisks). Notice the shrunken deeply stained nuclei of the granule cells in the granular layer (G) (curved arrows) (H&E x400).

Fig. 6: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIb showing Purkinje cells with fragmented nuclei (arrows). Another Purkinje cell having shrunken deeply stained nucleus (arrowhead) is seen. Notice a Purkinje cell with peripheral chromatin margination (wavy arrow) (H&E x400).
EFFECT OF METANIL YELLOW ON CEREBELLAR CORTEX AND THE POSSIBLE PROTECTIVE ROLE OF ANISE OIL

Fig. 7: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIb showing the Purkinje cells shrunken and distorted with irregular outlines (arrows), and they are surrounded by large prominent vacuolar spaces (asterisks) and many nuclei of neuroglia (arrow heads).

(H&E x400)

Fig. 8: A photomicrograph of a section in cerebellar cortex of metanil yellow and anise oil-treated subgroup IVa showing apparently normal cortical layers; molecular (M), Purkinje (P) and granular (G) layers. Most of Purkinje cells are more or less normal (arrows). A Purkinje cell (arrow head) is seen shrunken and darkly stained.

(H&E x400)

Fig. 9: A photomicrograph of a section in cerebellar cortex of metanil yellow and anise oil-treated subgroup IVb showing most of Purkinje cells are apparently normal (arrows). An irregular Purkinje cell (arrow head) is seen with darkly stained nucleus and cytoplasm and surrounded by unstained haloes (asterisks). Some vacuolar spaces (wavy arrows) in the molecular and granular layers are observed

(H&E x400)

Fig. 10: A photomicrograph of a section in cerebellar cortex of control group I showing a faint positive reaction for active caspase-3 in few cells (arrow heads) in the three cortical layers; molecular (M), Purkinje (P) and granular (G).

(Active caspase-3 x400)

Fig. 11: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIa showing a strong positive cytoplasmic immunoreaction in many cells in the 3 cortical layers; molecular (M), Purkinje (P) and granular (G) (arrows).

(Active caspase-3 x400)

Fig. 12: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIb showing apparent increase in the active caspase-3 positive cells in the three cortical layers; molecular (M), Purkinje (P) and granular (G) (arrows).

(Active caspase-3 x400)
Fig. 13: A photomicrograph of a section in cerebellar cortex of metanil yellow and anise oil-treated subgroup IVa showing a weak positive cytoplasmic immunoreaction in few cells in the Purkinje cell layer (P) (arrows).

(Active caspase-3 x400)

Fig. 14: A photomicrograph of a section in cerebellar cortex of metanil yellow and anise oil-treated subgroup IVb showing a moderate positive cytoplasmic immunoreaction in some cells in the Purkinje cell layer (P) (arrows).

(Active caspase-3 x400)

Fig. 15: A photomicrograph of a section in cerebellar cortex of control group I showing few astrocytes in the granular (G) and molecular layers (M) exhibiting a weak positive cytoplasmic immunoreaction (arrows).

(GFAP x400)

Fig. 16: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIa showing a strong positive cytoplasmic immunoreactions in many astrocytes in the three cortical layers; molecular (M), Purkinje (P) and granular (G) (arrows).

(GFAP x400)

Fig. 17: A photomicrograph of a section in cerebellar cortex of metanil yellow-treated subgroup IIIb showing apparent increase in the GFAP positive cells in the three cortical layers; molecular (M), Purkinje (P) and granular (G) (arrows).

(GFAP x400)

Fig. 18: A photomicrograph of a section in cerebellar cortex of metanil yellow and anise oil-treated subgroup IVa showing a moderate positive cytoplasmic immunoreaction in few astrocytes in the granular (G) and molecular (M) layers (arrows).

(GFAP x400)
EFFECT OF METANIL YELLOW ON CEREBELLAR CORTEX AND THE POSSIBLE PROTECTIVE ROLE OF ANISE OIL

DISCUSSION

Despite the wide spread use of metanil yellow as a synthetic food colorant, there is a growing concern about its many health hazards\(^\text{[32]}\). However, few studies on the effect of metanil yellow on the cerebellum were conducted\(^\text{[33]}\). Anise oil is reported to have protective effects against different cerebrovascular diseases and neurological disorders\(^\text{[34]}\). Accordingly, we aimed to study the effect of long term administration of metanil yellow on the structure cerebellar cortex of adult Albino rat and to evaluate the potential role of anise oil as a protective agent employing different histological and immunohistochemical methods.

In the present study, administration of metanil yellow caused evidence of structural changes in the rat cerebellar cortex mainly in the Purkinje cell layer. These changes reached its maximum at the 8th week of administration resulting in a significant decrease in the number of Purkinje cells that became damaged and lost. This goes hand to hand with another study that have reported a disorganized Purkinje cell layer and loss of Purkinje cells after metanil yellow administration\(^\text{[35]}\). Moreover, these findings were in accordance with previous studies that reported that chronic administration of metanil yellow predisposed the developing and adult rats to neurotoxicity attributed to alterations of the major neurotransmitter systems\(^\text{[36]}\).

In addition, darkly stained Purkinje cells and granule cells were observed in metanil yellow-treated group that seemed to be resulted from neuronal degeneration\(^\text{[37]}\). Other investigators explained the dark staining of degenerated neurons to be a consequence of an accumulation of denatured proteins resulting from failure of the antioxidant system with subsequent uncompensated oxidative stress\(^\text{[38]}\). On the other hand, these dark neurons were attributed to ischemia, or possibly due to abnormalities in the capillary wall of the cerebellar cortex with subsequent disorders in the structure of blood-brain barrier\(^\text{[39]}\).

In metanil yellow-treated group, the Purkinje cells and granule cells appeared with dark (pyknotic) nuclei. These pyknotic nuclei might be a result of an irreversible condensation of chromatin in cells undergoing programmed cell death or apoptosis\(^\text{[40]}\). In addition, in metanil yellow-treated rats, our results revealed a highly significant increase in the mean number of immunostained cells for active caspase-3, which is an apoptotic marker\(^\text{[41]}\). Thus, we suggest the apoptosis to be a mechanism of metanil yellow induced neurotoxicity. In addition, metanil yellow administration caused nuclear fragmentation of the affected Purkinje cells, and this is one of the apoptotic markers as reported by some authors\(^\text{[42]}\).

In metanil yellow-treated group, the Purkinje cells appeared distorted and shrunken, this might be due to damage in the system responsible for cell proteins biosynthesis. In addition, these shrunken Purkinje cells were surrounded by large prominent pericellular spaces, and this could be attributed to the shrinkage of neurons with subsequent withdrawal of their processes, due to disintegration of their cytoskeletal elements\(^\text{[43]}\).

In fact, the mechanism of metanil yellow induced neurotoxicity is not well known. However, many studies have reported oxidative stress to be the central mechanism of its toxicity, by promoting lipid peroxidation products and reactive oxygen species, and thus, inhibiting endogenous antioxidant defense enzymes and cause brain tissue damage\(^\text{[44, 45]}\).

The brain tissue contains relatively low levels of antioxidants and high levels of polyunsaturated lipids, which makes it more vulnerable to oxidative stress compared to other tissues\(^\text{[46]}\). Moreover, the brain tissue displays variations in the cellular and regional distribution of the antioxidant biochemical defenses\(^\text{[47]}\).

In this study, the mean percentage (%) of GFAP immunostained cells was highly significantly increased as compared with control group. GFAP is an intermediate filament protein known to be specifically expressed in astrocytes, the glial cells that are responsible for repairing and scarring of the brain following injuries\(^\text{[48, 49]}\). The increase in GFAP expression has been documented as a biomarker of neurotoxicity\(^\text{[50]}\), whereas exposure to any neurotoxic substances stimulates astrocytes’ proliferation and hypertrophy with subsequent increase in the synthesis of GFAP leading to vigorous astrogliosis which is a compensatory neuro-protective process\(^\text{[51, 52]}\).

At the protective level, anise oil co-administration with metanil yellow could to some degree preserve the
structure of the cerebellar cortex at the histological and immunohistochemical levels. This may indicate that anise oil has neuroprotective effect against metanil yellow-induced neurotoxicity. Alike, previous research reported the anise oil as a neuroprotective agent against the cerebellar structural changes induced by chronic administration of other toxic agents such as bisphenol A[50].

In addition, Kahloula and colleagues documented the beneficial effect of anise oil against a variety of neurological disorders such as depression, memory disorders, cerebral ischemia and Alzheimer disease[51]. Which might be due to the antioxidant potential of anise oil that protect against various causes of brain damage, and this may be related to its content of the bioactive ingredient; anethole[52] that is structurally related to dopamine and catecholamines. Noteworthy, anethole is a widely used substrate for synthesis of many drugs of neuro-pharmaceutical interest such as anti-convulsant and sedative drugs[53]. Others attributed the neuroprotective potential of anise oil to be through induction of neuronal excitability by activating the Ca2+ canals or inhibiting the Ca2+/K+ voltage dependent canal[54].

CONCLUSION

Based on our histological and immunohistochemical results, we can conclude that chronic administration of metanil yellow adversely altered the structure of cerebellar cortex of adult albino rats. So, it is necessary to increase the consumer awareness regarding the serious effects of chronic use of metanil yellow in diet, and to replace it with natural products. In addition, the concomitant administration of anise oil can minimize the hazardous effects of metanil yellow.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

14. Nagaraja TN and Desiraju T. Effects of chronic consumption of metanil yellow by developing and adult rats on brain regional levels of noradrenaline,


34. Fritsch P, Richard-Le Naour H, Denis S, Menetrier F. Kinetics of radiation induced apoptosis in the cerebellum of 14-day-old rats after acute or


الملخص العربي

التأثير طويل الأمد للميتانيل الأصفر على تركيب قشرة المخيخ لذكور الفئران البيضاء البالغة والدور الوقائي المحتمل لزيت اليانسون: دراسة هستولوجية وهستوكيميائية مناعية

ولاء محمد علوان
قسم الهستولوجى، كلية الطب، جامعة طنطا، طنطا، مصر

المقدمة:

الملخص العربى

يستخدم الميتانيل الأصفر في تلوين العديد من الصناعات الغذائية. إلا أن له آثار سامة على بعض الأعضاء في البشر والحيوانات. وتميز زيت اليانسون "الذي هو الزيت الأساسي للنباتات المزهرة بيمينيلا أنيسوم" بمعالجة واسعة من الأنشطة الدوائية كما أن له دور مفترض في مكافحة بعض الاضطرابات العصبية.

الهدف من البحث:

هدف هذا العمل إلى دراسة التأثير طويل الأمد للميتانيل الأصفر على تركيب قشرة المخيخ لذكور الفئران البيضاء البالغة وتقييم الدور الوقائي المحتمل لزيت اليانسون.

مواد وطرق البحث:

تم تقسيم خمسة وأربعون من ذكور الفئران البيضاء البالغة إلى أربع مجموعات: المجموعة الأولى (الضابطة)، المجموعة الثانية مقسمة إلى مجموعات فرعية أ و ب أعطيت زيت اليانسون 0.5 مل / كجم بالفم لمدة أربعة أسابيع، ومجموعة الثالثة مقسمة إلى مجموعات فرعية أ و ب أعطيت ميتانيل الأصفر 200 ملجم / كجم بالفم لمدة أربعة أسابيع، ومجموعة الرابعة مقسمة إلى مجموعات فرعية أ و ب أعطيت كلاً من زيت اليانسون وميتانيل الأصفر بنفس الجرعة والطريقة والمدة كما في المجموعتين الثانية والثالثة. تم تجهيز عينات من القشرة المخيخية للمجهر الضوئي. كما أجريت دراسة مناعية باستخدام أجسام مضادة لكاسبيز 3، والبروتين الليفي الحمضي للخلية الدبقية.

نتائج:

أظهرت عينات من الحيوانات التي أعطيت ميتانيل الأصفر انخفاضاً ذو دلالة إحصائية عالية في متوسط عدد خلايا البيركنجي، وظهرت خلايا بيركنجي مشوهة ومتقلصة ومحاطة بمساحات فراغية كبيرة. وكانت بعض أنواعها متقلصة ولونها بقع، وظهورها مجزأة. كما أظهرت الطرق الجزيئية والحيضوية فراغات بارزة حول الخلايا العصبية. على المستوى الهستوكيميائي المناعي، فقد لوحظت زيادة ذات دلالة إحصائية عالية في نشاط كاسبيز 3، والبروتين الليفي الحمضي للخلايا الدبقية، وفي المقابل: لوحظت تغيرات ضئيلة في الفئران التي أعطيت ميتانيل الأصفر بالتزامن مع زيت اليانسون.

الاستنتاج:

تشبب ميتانيل الأصفر في حدوث تغيرات تركيبية في قشرة المخيخ للفئران البالغة التي يمكن أن تتحسن بالعلاج المصاحب بزيت اليانسون.