In Vivo and *in Vitro* Studies on the Effect of Colchicine and Possible Protective Role of Lithium on Cerebellar Cortex Postnatally in Albino Rat offsprings.

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Abstract: Colchicine is a widely used drug in certain diseases and has many side effects. This work was carried out to study the effect of colchicine (4 mg/kg body weight) single dose on the cerebellar cortex of albino rat offisprings and the possible protective role of lithium chloride at ages of 15 and 28 days old rats and in vitro through making tissue cultures obtained from the cerebellar cortex of 7-days old rats Fifty neonate albino rats for in vivo studies and twenty five cultures were used in this study. The rats in the in vivo studies and the cultures of the in vitro studies were divided into three groups, as control group, colchicine treated group and colchicine with lithium chloride treated group. Sections of the cerebellar cortex were subjected to light microscopic, immunohistochemical, statistical and the cultures were subjected to genetic studies. Administration of colchicine revealed that, the sections of the cerebellar cortex of treated rats showed disruption and cellular degenerative changes of the Purkinje and the granular cells. Improvement of the histology of the cerebellar cortex occured with administration of lithium chloride. These findings indicate that, colchicine consumption may have some deleterious effects on the cerebellum of the neonates and may affect the functions of the cerebellum leading to tremors, unstable, uncoordinated movements and ataxia. So, it is recommended that colchicine should be used with precautions and in a minimal dose and with concurrent use of lithium chloride.

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1. Introduction

Cytoskeletal alteration is a key factor in neurodegenerative processes like Alzheimer's or Parkinson's disease. Understanding of the mechanism of neuronal cell death is vital to synthesize drugs for the treatment of neurological disorder**.** It is widely accepted that apoptosis is the main process implicated in neuronal cell death in the majority of the neurodegenerative diseases **(Pallas *et al.*, 2006)**.

One characteristic common to all neurodegenerative diseases is oxidative stress production at the mitochondrial level. DNA damage is also involved in the process of neuronal cell death. DNA damage is a target of oxidative stress **(Copani *et al.*, 2007).**

Mitochondria play a prominent role in the apoptotic pathway involved in neuronal cell death because they release several key proteins such as cytochrom C and the apoptotsis inducing factor (AIF) to the cytoplasm. In addition cytochrom C participates in apoptosis by activating the caspase dependent pathway while AIF induces cell death by a capase-independent pathway **(Volbracht *et al.*, 2001).**

Colchicine is a microtubule disrupting agent that binds to tubuline, inhibiting microtubule assembly which triggers apoptosis. Colchicine triggers apoptosis in several neurons in the in vitro models such as granular neuronal culture **(Kristensen *et al.*, 2003).**

Mood stabilizing drugs such as lithium, used in the treatment and prophlaxis of bipolar disorders, show neuroprotective effects against colchicine induced apoptosis in rat cerebellar granular neurons (CGN) **(Alvarez *et al.*, 1999).**

This work demonstrates that the activation of caspase-3 by colchicine is inhibited in the presence of lithium. All these findings confirm earlier reports and are in line with evidence that lithium chloride exerts wide neuroprotective and anti-apoptotic effects in a number of neuronal models **(Hennion *et al.*, 2002).**

The aim of this work is to evaluate the possible protective role of a mood stabilizer, lithium chloride, against colchicine induced apoptosis in rat cerebellum by histological, immunohistochemical and DNA. analytic studies.

2. Material and Methods

1- Animals:

Thirty pregnant mothers of albino rats were obtained from Helwan Animal House. Their offsprings were kept on breast feeding with their

mothers and with standard diet in a healthy conditions during the whole experimental period. Fifty 7-days old albino rat offisprings were used for *in vivo* studies with average weight 30 grams, and twenty five primary cultures of cerebellar granular neurons (CGNs) were also prepared from 7-days old albino rat offisprings following the method of **Verdaguer *et al.*, 2002.**

2- Chemicals:

Colchicine:

It was obtained from Sigma medical company of Pharmaceutical Industries as a powder and dissolved in distilled water (D.W).

Lithium chloride:

It was obtained as a powder from EL-Gomhoria company of Pharmaceutical Industries and was dissolved in distilled water (D.W).

Fetal calf serum:

It was obtained from Sigma company, USA.

Cytosine arabinoside:

It was obtained from Sigma company of Pharmaceutical Industries

Monoclonal mouse caspase-3 antibody:

It was obtained from Sigma Co. of Pharmaceutical industries as a liquid. The antibody was exposed to microwave treatment for 10 minutes in a citrate buffer.

3- Experimental design:

A) *In vivo* studies:

Fifty offsprings of albino rats aged seven days old were used in this study. Their average weight was 30 grams. They were divided into the following groups:

1- Control group (A): thirty offsprings of albino rats, 7-days old were used and divided into two subgroups:

a) Negative control subgroup (subgroup A1): was consisted of ten offsprings received nothing allover the experimental period. After 7 days (one week), five of them were anaesthetized lightly with ether then sacrificed, the other five were sacrificed after 3 weeks by the same method.

b) Positive control subgroup (subgroup A2): was consisted of twenty offsprings and divided as follows:

1- Vehicle control subgroup: was consisted of ten offsprings, each of them was treated with 1 ml distilled water (the solvent of colchicine) intraperitoneally for one day then no treatment was given. After 7 days, five of them were anaesthetized lightly with ether then sacrificed, the other five were sacrificed after 3 weeks by the same method.

2- Lithium chloride treated control subgroup: was consisted of ten offsprings, each of them was treated only with lithium chloride 40 mg/kg body weight dissolved in 1 ml distilled water intraperitoneally for 7 days **(Chuang *et al.*, 2002).** After these 7 days, five of them were anaesthetized lightly with ether then sacrificed, the other five were sacrificed after 3 weeks by the same method.

2- Colchicine treated group (B): was consisted of ten 7-days old rats, they received 4 mg/kg body weight colchicine dissolved in 1ml distilled water by a single intraperitonial injection **(Masako *et al.*, 2000).** After 7 days, five of them were anaesthetized lightly with ether then sacrificed, the other five were sacrificed after 3 weeks by the same method.

3- Lithium chloride and Colchicine treated group (Protected group) (C): was consisted of ten, 7-days old rats, they received 4 mg/kg body weight colchicine dissolved in 1ml distilled water by a single intraperitonial injection, then they received daily dose of lithium chloride 40mg/kg body weight dissolved in 1 ml distilled water intraperitonially for 7 days **(Masako *et al.*, 2000 & Chuang *et al.*, 2002).** After these 7 days, five of them were anaesthetized lightly with ether then sacrificed, the other five were sacrificed after 3 weeks by the same method.

The rats were anaesthetized lightly by diethyl ether inhalation as above, then sacrificed and their brains were extracted then the cerebella were separated and preserved in Bouin's solution. Then paraffin sections were prepared. The thickness of sections used was 5 microns.

- Methods for *in vivo* studies:

Rats were sacrificed according to the above periods. The rats were anaesthetized lightly by diethyl ether inhalation and their brains were extracted then the cerebella were separated and preserved in Bourns solution. Then paraffin sections were prepared. The thickness of sections used was 5 microns.

*Histological study, using haematoxylin and eosin staining. Also by toluidine blue staining.

*Immunohistochemical study, using caspase-3 enzyme.

*statistical techniques.

B) In vitro studies:

Twenty five offsprings of albino rats aged seven days old were used for culture preparation according to the method of **Verdaguer *et al.*, 2002.** They were divided into the following groups:

1- Control group (A): Fifteen cultures were formed in this group from 7 days old albino rat offsprings and divided into two subgroups:

a) Negative control subgroup (subgroup A1): contained five cultures obtained from 7 days old albino rat offsprings. After 7 days in vitro, CGNs cultures were kept without any additions and

served as control for all *in vitro* experimental groups.

b) Positive control subgroup (subgroup A2): contained ten cultures and divided as follows:
   1- Vehicle control subgroup: contained five cultures obtained from 7 days old albino rat offsprings. After 7 days *in vitro*, CGNs cultures were incubated for 24 hours in complete medium containing 5 ml distilled water.
   2- Lithium chloride treated control subgroup: contained five cultures obtained from 7 days old albino rat offsprings. After 7 days *in vitro*, CGNs cultures were incubated for 24 hours in complete medium containing lithium chloride (5mM) **(Jorda *et al.*, 2004).**

2- Colchicine treated group (B): contained five cultures obtained from 7-days old rats. After 7 days *in vitro*, CGNs cultures were incubated for 16 hours in medium containing colchicine (1mM) **(Jorda *et al.*, 2004).**

3- Lithium chloride and Colchicine treated group (C): contained five cultures obtained from 7 days old rats. After 7 days *in vitro*, CGNs cultures were incubated for 24 hours in complete medium containing lithium chloride (5mM) then colchicine (1 mM) was added **(Jorda *et al.*, 2004).**

- Methods for *in vitro* studies:

In each group, the cultures were subjected to D.N.A. analytic studies by gel electrophoresis. Preparation of the culture were done through the method of **Verdaguer *et al.*, 2002.**

3. Results

In this study it was found that in the vehicle & lithium chloride treated subgroups, the results were the same as that of control group.

At 15th day old age

I- In vivo studies:

1- Haemoatoxylin & Eeosin stained sections:

The cerebellar cortex of control group consisted of four layers: the external granular layer, the molecular layer, the purkinje cell layer and the internal granular layer (Fig. 1).

The cerebellar cortex of **control group** showed that, the external granular layer formed of a sheet of closely packed cells of 4-5 cell layers. The superficial cells were closely packed together, while the deeper cells were separated from each other and were arranged perpendicular to the outer surface (Fig. 1). Both the superficial and the deep cells were oval or rounded in shape with deeply stained nuclei. The molecular layer appeared as a narrow zone beneath the external granular layer. The Purkinje cells arranged as one layer of flask shaped cells between the thin molecular layer and the internal granular layer. The internal granular cell layer showed oval and rounded shapes with lightly stained nuclei (figs. 2 & 3).

The cerebellar cortex of **Colchicine treated group** showed that, the external granular layer was more or less normal as compared with the control one. It appeared thicker than the control group. The molecular cell layer was more thinner than the control group (less developed) (Fig. 4). The purkinje cells appeared small and less developed than the control one. In some areas, there was disruption of the arrangement of the purkinje cells and there were areas of purkinje loss (fig. 5). The cells of the internal granular layer were smaller in size compared with the control group and deeply stained (fig. 5 & 6).

The mean thickness of the molecular layer of rats receiving colchicine was *2.7 ± 0.6* compared with *5.9 ± 1.1* in control group. There was significant decrease in the thickness of the molecular layer in rats receiving colchicine compared with control group (P value < 0.05) as shown in **table (1)** and **column chart (1).**

The mean number of the Purkinje cells of rats receiving colchicine was 5.0 ± 2.3 compared with 16.0 ± 4.4 in control group. There was significant decrease in the number of the Purkinje cells in rats receiving colchicine compared with control group (*P* value < 0.05) as shown in **table (2)** and **column chart (2).**

The layers of the cerebellar cortex in **protected group** were more or less as the control group. The external granular layer was formed of a sheet of closely packed cells of 4-5 cell layers. The molecular layer appeared as a narrow zone beneath the external granular layer. The Purkinje cells arranged as one layer between the thin molecular layer and the internal granular layer with the appearance of normal rounded to flask shaped cells beside the degenerated ones (Figs. 7 &8). The internal granular layer showed small rounded or oval cells.

The mean thickness of the molecular layer of the group receiving colchicine + lithium chloride was 4.3 ± 1.0. There was significant increase in the thickness of the molecular layer in rats receiving colchicine + lithium chloride compared with the colchicine treated group (*2.7 ± 0.6)* (*P* value was < 0.05) as shown in **table (1)** and **column chart (1).**

The mean number of the Purkinje cells of the group receiving colchicine + lithium chloride was 9.0 ± 1.6. There was significant increase in the number of Purkinje cells in rats receiving colchicine + lithium chloride compared with the colchicine treated (5.0 ± 2.3, P value < 0.05) as shown in **table (2)** and **column chart (2).**

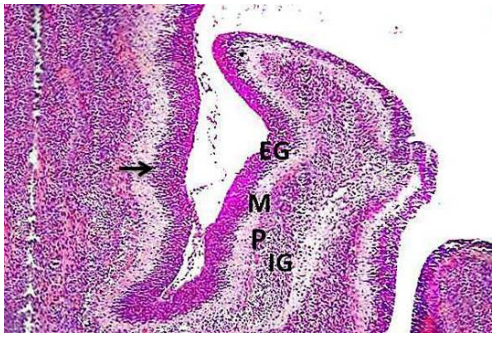


Fig (1): A Photomicrograph of a section of the cerebellar cortex of 15 days old albino rat offispring showing the layers of the cerebellar cortex. It is composed of an external granular layer (EG) with its deep cells perpendicular to the surface (arrow), a molecular layer (M), a Purkinje cell layer (P) and an internal granular layer (IG). **(Hx & E X 100).**

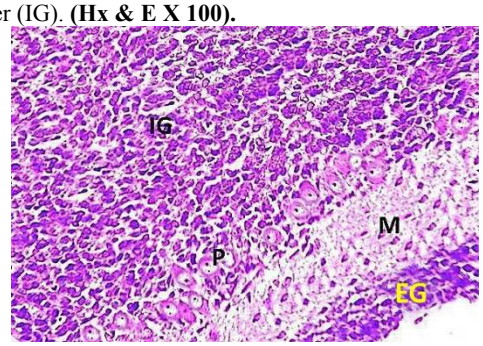


Fig (2): A Photomicrograph of a section of the cerebellar cortex of a control rat aged 15 days showing the external granular layer (EG). It is formed of a sheet of closely packed cells of 4-5 cell layers, the cell are round or oval with deeply stained nuclei. The molecular layer (M) is a thin rim between the external granular layer and the purkinje cell layer. The Purkinje cells (P) are flask shaped with well defined nuclei. They are arranged as a mono cellular layer of round to flask shaped cells at the outer margin of the internal granular layer. The internal granular layer (IG) formed of small rounded cells. **(Hx & E X 400).**

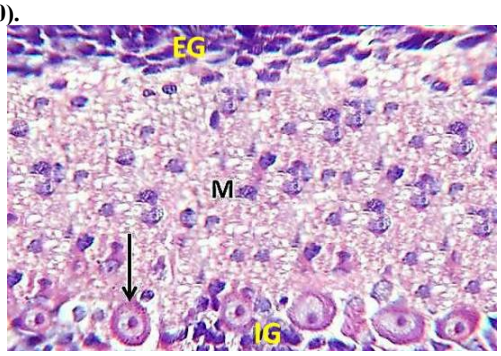


Fig (3): A Photomicrograph of a section of the cerebellar cortex of a control rat aged 15 days showing the layers of the cerebellar cortex: external granular layer (EG), a molecular layer (M), the Purkinje cells (arrow) each has big rounded vesicular nucleus with deeply stained cytoplasm and an internal granular layer (IG). **(Hx & E X 1000, oil immersion).**

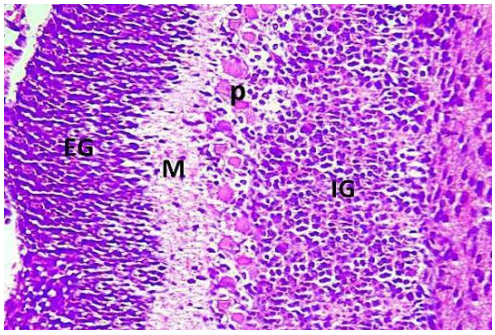


Fig (4): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 15 days showing increased thickness of the external granular layer (EG) with its inner cells are perpendicular to the cortical surface and the molecular layer (M) is thinner than that of the control group. The Purkinje cells (P) appear small in size with darkely stained pykontic nucleui and they are disarranged. The internal granular layer cells (IG) show small rounded deeply stained nuclei. **(Hx & E X 400).**

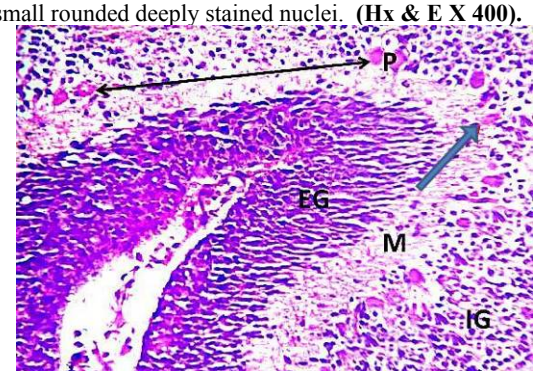


Fig (5): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 15 days showing increased thickness of the external granular layer (EG) with decreased thickness of the molecular layer (M). The Purkinje cells (P) are degenerated and shrunken (thick blue arrow) with areas of purkinje loss (thin black arrow). The internal granular cells (IG) are small and widely separated. **(Hx & E X 400).**

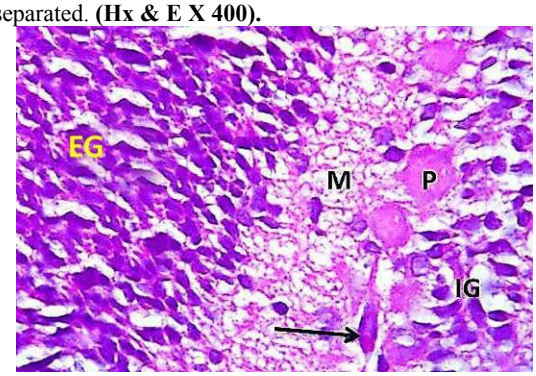


Fig (6): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 15 days showing the layers of the cerebellar cortex: external granular layer (EG), a molecular layer (M), the Purkinje cell layer cells (P), which appear degenerated and shrunken (arrow) with deeply stained pyknotic nuclei and an internal granular layer (IG). **(Hx & E X 1000, oil immersion).**

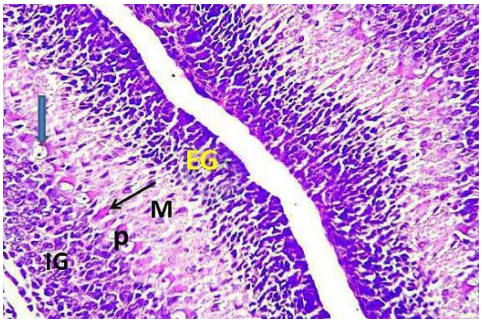


Fig (7): A photomicrograph of a section of the cerebellar cortex of a 15 days aged rat treated with colchicine + lithium chloride showing a picture more or less than the control group; the four layers of the cerebellar cortex. The external granular layer (EG), the molecular layer (M) and the Purkinje cell layer (P) is arranged as mono cellular layer at the outer margin of the internal granular layer. Normal Purkinje cells (thick blue arrow) are rounded to flask in shape with the degenerated and shrunken cells (thin black arrow). Note the internal granular layer (IG) **(Hx &E X 400).**

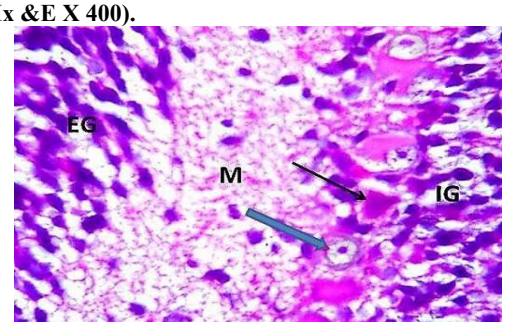
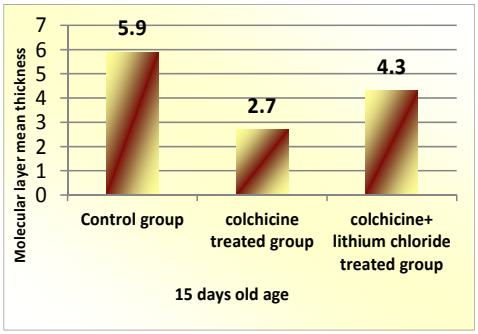


Fig (8): A photomicrograph of a section of the cerebellar cortex of a 15 days aged rat treated with colchicine + lithium chloride showing the layers of the cerebellar cortex: an external granular layer (EG), a molecular layer (M), the Purkinje cells (thick blue arrow) which are nearly normal in size and shape and shows the characteristic flask shaped appearance There are also degenerated purkinje cells (thin black arrow). Note the internal granular layer (IG). **(Hx&E X 1000, oil immersion)**

Table (1): Showing mean thickness of the molecular layer of 15 days old rats:

Age	Control group x±SD	colchicine treated group x±SD	colchicine+ lithium chloride treated group	Mannwhitney (M) Test	*P* value
15 days old	5.9±1.1	2.7±0.6	4.3± 1.0	M1= 2.6 M2= 2.2 M3= 2.3	*P*1<0.01 *P*2<0.05 *P*3<0.05

(X: mean and SD: standard deviation**).**
- *P* value ≤ 0.05: significant.
- *P* value ≤ 0.001: highly significant.
- *P* value > 0.05: non significant.
-*P*1&M1= the relation between colchicine treated and control groups.
-*P*2&M2=the relation between colchicine + lithium chloride and control groups.
-*P*3&M3= the relation between colchicine + lithium chloride and colchicine treated groups.

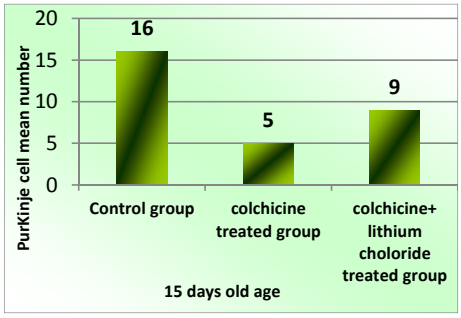


C***olumn chart (1):*** Comparison between the control, colchicine treated, and colchicine + lithium chloride groups of rats at 15 days regarding the thickness of molecular layer

Table (2): Showing mean number of the Purkinje cells of 15 days old rats:

Age	Control group x±SD	colchicine treated group x±SD	colchicine+ lithium choloride treated	Mannv (M) Te
15 days old	16.0±4.4	5.0±2.3	9.0±1.6	M1= 2. M2= 2. M3= 2.

(X: mean and SD: standard deviation**).**
P value ≤ 0.05: significant.
P value ≤ 0.001: highly significant.
P value > 0.05: non significant.
*P*1&M1= the relation between colchicine treated and control groups.
*P*2&M2= the relation between colchicine + lithium chloride and control groups.
*P*3&M3= the relation between colchicine + lithium chloride and colchicine treated groups.



Column chart (2): Comparison between the control, colchicine treated, and colchicine + lithium chloride groups of rats at 15 days regarding the number of Purkinje cells.

2- Toluidine blue stained sections:

The cerebellum of the control group was found to be consisted of the four layers of the cerebellar cortex: the external granular layer, the molecular layer, the Purkinje cell layer and the internal granular layer and the cerebellar medulla (Fig. 9).

In control sections, the external granular layer was dark blue and the purkinje cells were oval in shape with granular blue cytoplasm and arranged in one row. The cells of the molecular layer were dark blue. The cells of the internal granular layer were also deeply stained. The Purkinje cells were rounded to flask shaped and dark blue. The blue staining of all cells was due to the presence of Nissl's granules (Figs. 10 &11).

In colchicine treated sections, the external granular layer was paler than the control group. The Purkinje cells were shrunken and irregular with ill defined nuclei and showed decrease in the intensity staining for Nissl's granules. The internal granular cells appeared pale blue (Figs. 12 &13).

In protected sections, the external granular layer was darker than colchicine treated sections. The Purkinje cell bodies were looked like control group in shape with increase in the intensity of staining for Nissl's granules as compared with colchicine treated sections. The internal granular cells were darker blue stained than colchicine treated sections (Figs. 14 &15).

3-Immunhistochemical (caspase-3) stained sections:

Examination of control sections revealed that, the cerebellar cortex showed negative immune reaction to caspase-3 (Fig. 16).

The cerebellar cortex of colchicine treated sections showed positive reaction to caspase-3 with appearance of dark brown areas especially the Pukinje cells. There was severe disruption of the purkinje cell shape and arrangement (Fig. 17).

In protected sections, there was slight increase in the brown staining of the cells of the cerebellar cortex compared with control sections but less than what was seen in the colchicine treated group (Fig. 18).

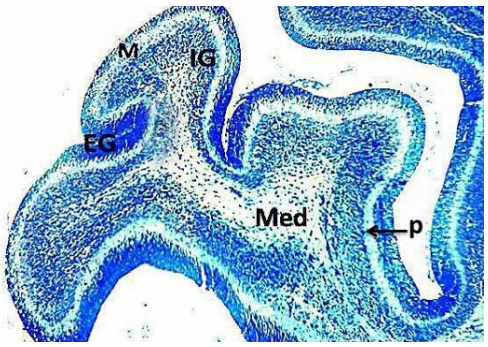


Fig (9): A Photomicrograph of a section in an albino rat cerebellum 15 days old showing the layers of the cerebellar cortex. It is composed of an external granular layer (EG), a molecular layer (M),a Purkinje cell layer (P) and an internal granular layer (IG) with the appearance of the cerebellar medulla (Med). **(Toluidine blue X 100).**

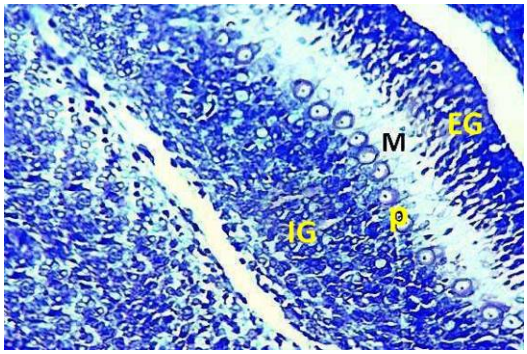


Fig (10): A photomicrograph of a section of the cerebellar cortex of control rat aged 15 days showing dark blue staining of Nissl's granules in the cytoplasm of the cells of the four layers of the cerebellar cortex; the external granular layer (EG), the molecular layer (M), the Purkinje cells (P) which are rounded to flask shaped with dark blue cytoplasm and the internal granular layer (IG). **(Toluidine blue X 400).**

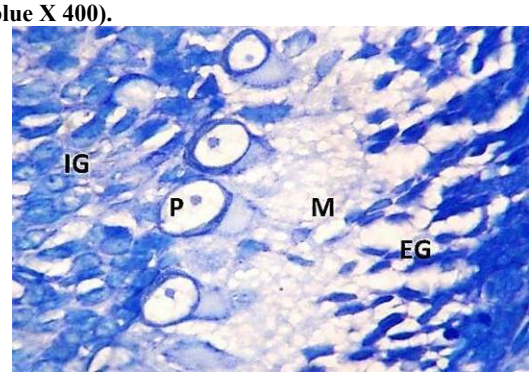


Fig (11): A photomicrograph of a section of the cerebellar cortex of control rat aged 15 days showing the layers of the cerebellar cortex: external granular layer (EG), a molecular layer (M), the Purkinje cells (P) which appear rounded each having big vesicular nucleus with a prominent deeply stained nucleolus and surrounded by Nissl's granules and an internal granular layer (IG). The granular cells are closely packed cells, each has oval deeply stained nucleus and scanty cytoplasm.**(Toluidine blue X 1000, oil immersion).**

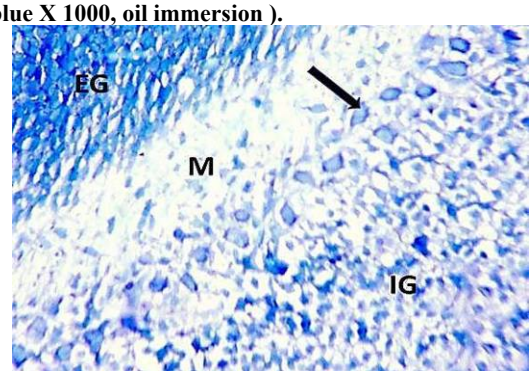


Fig (12): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 15 days showing decrease in the intensity staining for Nissl's granules of the external granular cells (EG), the molecular layer (M), the Purkinje cells, which are disarranged (arrow). Note the internal granular cells (IG). **(Toluidine blue x 400).**

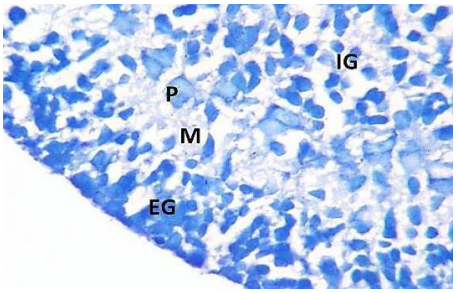


Fig (13): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 15 days showing shrunken and irregular Purkinje cells (P) which are disarranged, their nuclei are ill defined. There is decrease in the intensity staining for Nissl's granules in all layers of the cerebellar cortex: the external granular layer (EG), the molecular layer (M), the Purkinje cells and the internal granular layer (IG). **(Toluidine blue x 1000, oil immersion).**

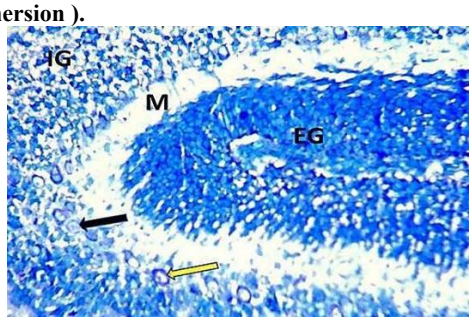


Fig (14): A photomicrograph of a section of the cerebellar cortex of a rat aged 15 days treated with colchicine + lithium chloride showing increase in the intensity staining for Nissl's granules compared with colchicine treated group in the four layers of the cerebellar cortex; the external granular layer (EG), the molecular layer (M), the Purkinje cells where some of them appear like control (yellow arrow) and some show decreased intensity staining for Nissl's granules (black arrow). Note the internal granular layer (IG). **(Toluidine blue x 400).**

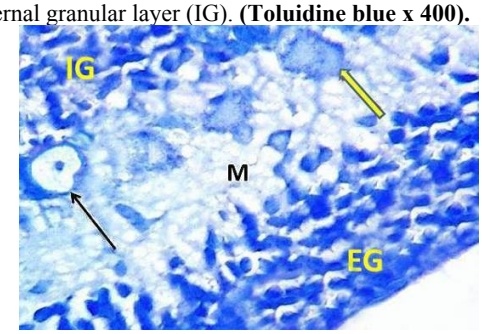


Fig (15): A photomicrograph of a section of the cerebellar cortex of a rat aged 15 days treated with colchicine + lithium chloride showing the layers of the cerebellar cortex: external granular layer (EG), a molecular layer (M), the Purkinje cells with a picture more or less similar to control group. The Purkinje cell appear like control with increased intensity staining for nissl's granules & vesicular nucleus (thin black arrow) beside the degenerated & lightly stained one's (thick yellow arrow). An internal granular layer (IG) **(Toluidine blue x 1000, oil immersion).**

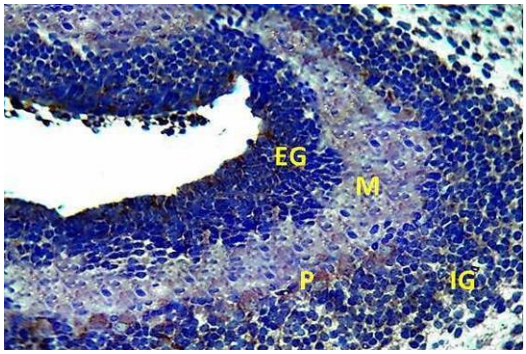


Fig (16): A Photomicrograph of a section of the cerebellar cortex of a control rat aged 15 days showing negative immune reaction to caspase-3 in the cytoplasm of the cells of the cerebellar cortex. Negative reaction is indicated by the absence of brown staining in the cytoplasm of the external granular (EG), the molecular (M), the Purkinje (P) and the internal granular cells (IG). **(Immunoreactivity to caspase-3 x 400).**

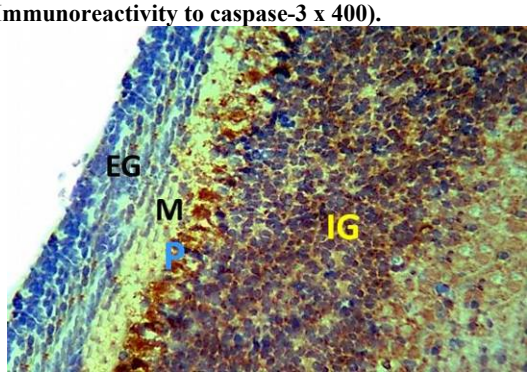


Fig (17): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 15 days showing positive immune reaction to caspase-3 in the cells of the cerebellar cortex. This is indicated by dark brown staining of the external granular cells (EG), the molecular layer (M), the Purkinje cells (P) which are disarranged and poorly developed and the internal granular cells (IG). **(Immunoreactivity to caspase-3 x 400**)

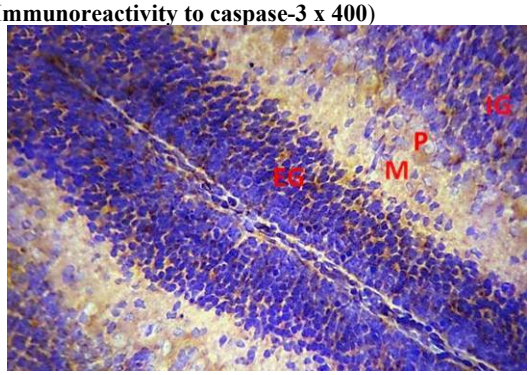


Fig (18): A photomicrograph of a section of the cerebellar cortex of a 15 days aged rat treated with colchicine + lithium chloride showing weak positive immune reaction to caspase-3 (light brown) in the cytoplasm of the cells of the external granular (EG), the molecular (M), the Purkinje (P) and the internal granular cells (IG). **(Immunoreactivity to caspase-3 x 400).**

At 28th day old age

1- Haemoatoxylin & Eeosin stained sections:

The cerebellar cortex of the control group at this age was found to be consisted of three layers: the molecular layer, the Purkinje cell layer and the granular layer (Fig. 19).

In control sections, the cerebellar cortex showed that, the external granular layer disappeared. The molecular layer became more apparent with increase in thickness. It contained variable number of fusiform and rounded-shaped cells of different sizes with darkly stained nuclei (Fig. 20).

The Purkinje cells increased in size and arranged in a single row superficial to the granular layer. The Purkinje cell bodies were oval or flask shaped with big rounded nuclei. The granular layer increased in thickness with rounded and oval cells of variable sizes (Fig. 21).

In colchicine treated sections, There was disarrangement of the Purkinje cells with loss of their characteristic flask shape (Fig. 22). They became ill-defined in shape. The majority of the Purkinje cells had pale homogenous cytoplasm with complete degeneration and darkly stained degenerated nuclei (Figs. 22 &25). There was, in some areas, focal loss of the Purkinje cells (Fig. 23). The cells of the granular layer appeared small rounded in shape and darkly stained (Figs. 22 & 23). There was persistence of the external granular layer, so the cortex was still formed of four layers in some areas (Fig. 24).

The mean thickness of the molecular layer of rats receiving colchicine was 5.3 ± 0.8 compared with 12.9 ± 2.5 in control group. There was significant decrease in the thickness of the molecular layer in rats receiving colchicine compared with control group ($P < 0.05$) as shown in **table (3)** and **column chart (3).**

The mean number of the Purkinje cells of rats receiving colchicine was 4.2 ± 2.4 compared with 11.0 ± 1.2 in control group. There was significant decrease ($P< 0.05$) in the number of the Purkinje cells in rats receiving colchicine compared with control group (**Table 4 a**nd **column chart 4).**

In protected sections, the external granular layer was completely absent. The majority of the purkinje cells bodies were oval or flask- shaped with big rounded nuclei with the appearance of normal rounded cells beside the diseased ones. The granular cells were rounded and darkly stained (Figs. 26 &27).

The mean thickness of the molecular layer of the group receiving colchicine + lithium chloride was 7.0 ± 1.3. There was significant increase $P< 0.05$ in the thickness of the molecular layer in rats receiving colchicine + lithium chloride compared with the colchicine treated group (5.3 ± 0.8) $P< 0.05$ (**Table 3 a**nd **column chart 3).**

The mean number of the Purkinje cells of the group receiving colchicine + lithium chloride was (8.0 ± 1.4). There was significant increase ($P< 0.05$) in the number of purkinje cells in rats receiving colchicine + lithium chloride compared with the colchicine treated group (4.2 ± 2.4) as shown in **table (4)** and **column chart (4).**

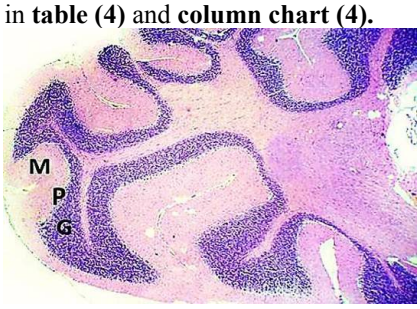


Fig (19): A Photomicrograph of a section in a control albino rat cerebellum 28 days old showing the layers of the cerebellar cortex. It is composed of a molecular layer (M), a Purkinje cell layer (P) and a granular layer (G). **(Hx & E X 100).**

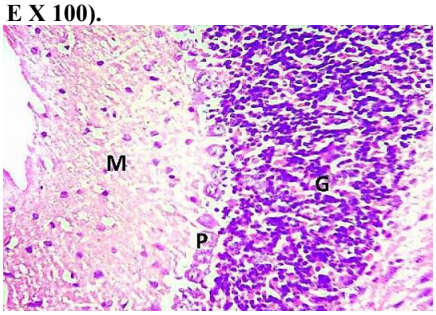


Fig (20): A photomicrograph of a section of the cerebellar cortex of a control rat aged 28 days showing the external granular layer disappeared. The molecular layer (M) became more apparent with increase in thickness. The Purkinje cells (P) increased in size and arranged in a single row superficial to the granular layer. The Purkinje cell bodies were oval or flask shaped with big rounded nuclei. The granular layer (G) increased in thickness with rounded and oval cells of variable sizes **(Hx & E X400).**

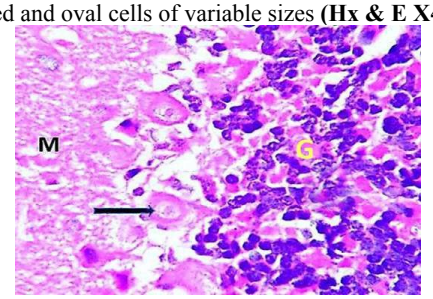


Fig (21): A photomicrograph of a section of the cerebellar cortex of a control rat aged 28 days showing the layers of the cerebellar cortex. It is composed of the molecular layer (M), the Purkinje cells (arrow) each having big rounded vesicular nucleus with deeply stained cytoplasm. The granular layer (G) consists mainly of numerous small closely packed deeply stained granular cells. **(Hx & E X1000, oil immersion).**

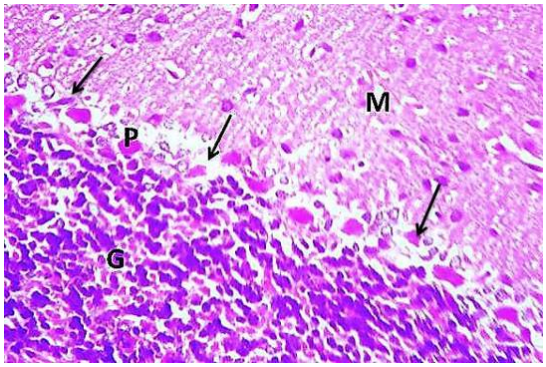


Fig (22): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 28 days showing the molecular layer (M). There are degenerated, shrunken and darkly stained Purkinje cells (P) (arrows). The granular cells (G) are also darkly stained. **(Hx & E X 400).**

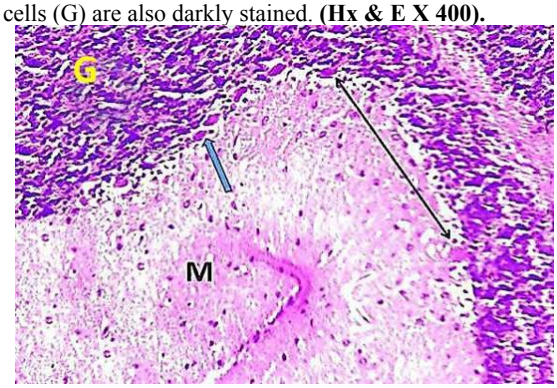


Fig (23): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 28 days showing the molecular cell layer (M) and areas of focal loss of the Purkinje cells (thin black arrow) and small degenerated Purkinje cells with loss of their characteristic flask shape (thick blue arrow). The granular cell layer (G). **(Hx & E X 400).**

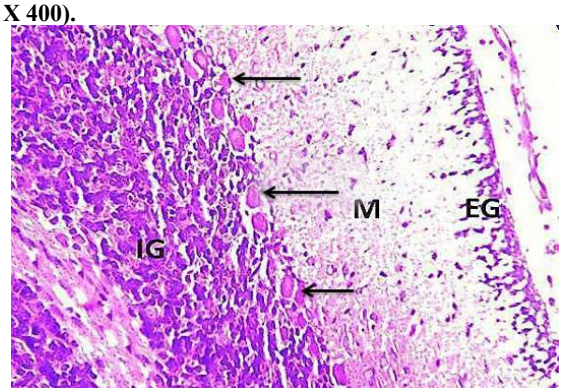


Fig (24): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 28 days showing decrease in the thickness of the molecular layer (M) as compared with the control group. Also there is persistence of the external granular layer (EG) in some areas, accompanied with the degenerated Purkinje cells (arrows). The internal granular layer (IG). **(Hx & E X 400).**

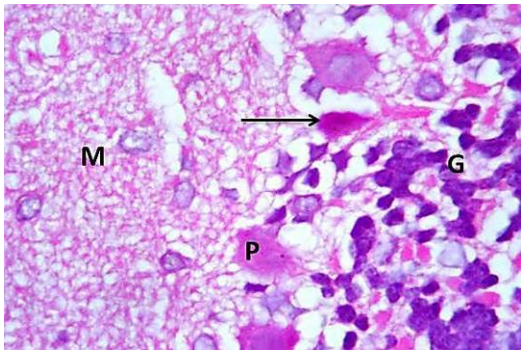


Fig (25): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 28 days showing the molecular layer (M). The degenerated Purkinje cells (P) which appear shrunken (arrow) with deeply stained pyknotic nuclei. The granular layer cells (G) have darkly stained nuclei. **(Hx & E X 1000, oil immersion).**

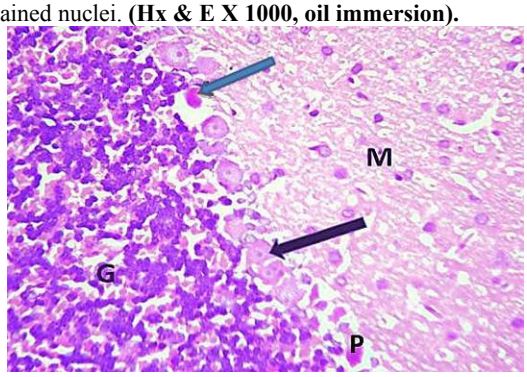


Fig (26): A photomicrograph of a section of the cerebellar cortex of rat aged 28 days treated with colchicine + lithium chloride showing the molecular layer (M) and the granular layer (G) are nearly like control. There is improvement in the Purkinje cells (P). The majority of them looks more or less like control ones (black arrow). Few of them are degenerated, deformed and shrunken (blue arrow). **(Hx & E X 400).**

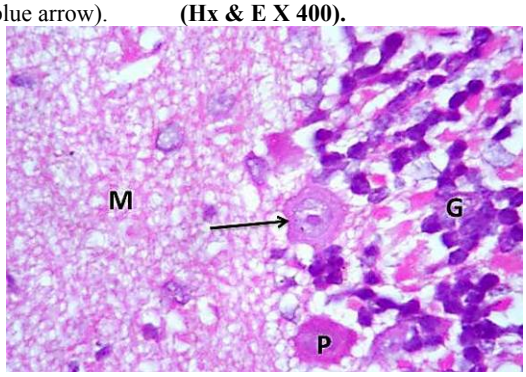
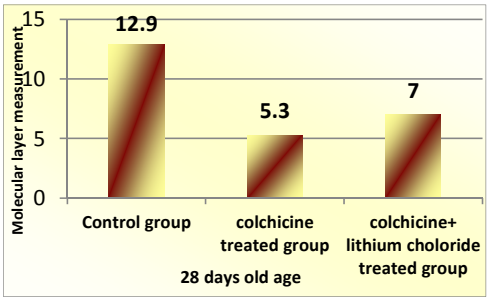


Fig (27): A photomicrograph of a section of the cerebellar cortex of rat aged 28 days treated with colchicine + lithium chloride showing the layers of the cerebellar cortex. It is composed of a molecular layer (M), a granular layer (G) and the Purkinje cell (arrow) nearly normal in size and shape and shows the characteristic rounded appearance with vesicular nucleus. There are also degenerated Purkinje cells (P) with darkly stained pyknotic nucleus. **(Hx & E X 1000, oil immersion).**

Table (3): Showing mean thickness of the molecular layer of 28 days old rats:

Age	Control group x±SD	colchicine treated group x±SD	colchicine+ lithium choloride treated group	Mannwhitney (M) Test	P valu
28 days old	12.9±2.5	5.3±0.8	7.0±1.3	M1= 2.6 M2= 2.6 M3= 2.2	P1<0.01 P2<0.01 P3<0.0

(X: mean and SD: standard deviation**).**
P value ≤ 0.05: significant.
P value ≤ 0.001: highly significant.
P value > 0.05: non significant.
*P*1&M1= the relation between colchicine treated and control groups.
*P*2&M2= the relation between colchicine + lithium chloride and control groups.
*P*3&M3= the relation between colchicine + lithium chloride and colchicine treated groups.



Column chart (3): Showing comparison between the control, colchicine treated, and colchicine + lithium chloride groups of rats at 28 days regarding the thickness of molecular layer.

Table (4): Showing mean number of the purkinje cells of 28 days old rats:

Age	Control group x±SD	colchicine treated group x±SD	colchicine+ lithium choloride treated group	Mannwhitney (M) Test	*P* value
28 days old age	11.0±1.2	4.2±2.4	8.0±1.4	*M1*= 2.6 *M2*= 2.4 *M3*= 2.2	*P1<0.01* *P2<0.05* *P3<0.05*

(X: mean and SD: standard deviation**).**
P value ≤ 0.05: significant.
P value ≤ 0.001: highly significant.
P value > 0.05: non significant.
*P*1&M1= the relation between colchicine treated and control groups.
*P*2&M2= the relation between colchicine + lithium chloride and control groups.
*P*3&M3= the relation between colchicine + lithium chloride and colchicine treated groups.

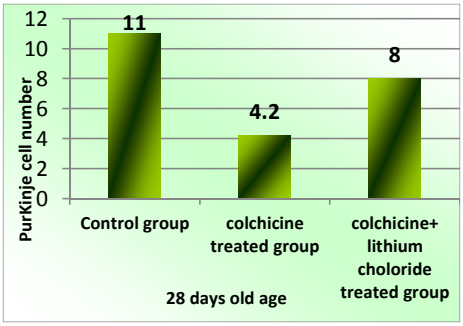
2- Toluidine blue stained sections:

The cerebellar cortex of the control group at this age was found to be consisted of three layers: the molecular layer, the Purkinje cell layer and the granular layer (Fig. 28).

In control sections, the Purkinje cells and the granular cells were dark blue due to the presence of Nissl's granules in the cytoplasm (Figs. 29 & 30).

In colchicine treated sections, the purkinje cells were small in size with decrease in Nissl's granules, so appeared lightly stained. Some of the granular cells appeared pale blue, the others appeared dark blue (Figs. 31 & 32).

In protected sections, the cytoplasm of some of the Purkinje and the granular cells showed increase in the intensity staining for Nissl's granules as compared with the colchicine treated group. Some of them were pale blue stained (Figs.33 &34).



Column chart (4): Showing comparison between the control, colchicine treated, and colchicine+lithium chloride groups of rats at 28 days regarding the number of Purkinje cells.

3- Immunohistochemical (caspase-3) stained sections:

In control sections: Examination of cerebellar cortex showed negative immune reaction to caspase-3(Fig. 35).

In colchicine treated sections: The cerebellar cortex showed positive reaction to caspase-3 with appearance of dark brown stained areas (Fig. 36).

In protected sections: There was slight increase in the brown staining of the cells of the cerebellar cortex compared with control group but was less than what was seen in colchicine treated group (Fig. 37).

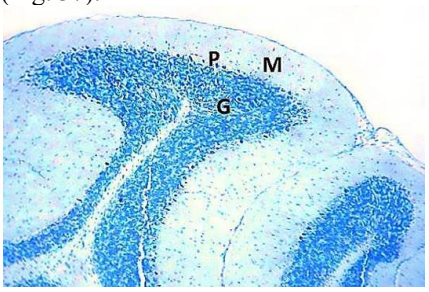


Fig (28): A Photomicrograph of a section in a control albino rat cerebellum 28 days old showing the layers of the cerebellar cortex. It is composed of a molecular layer (M), a Purkinje cell layer (P) and a granular layer (G). **(Toluidine blue X 100).**

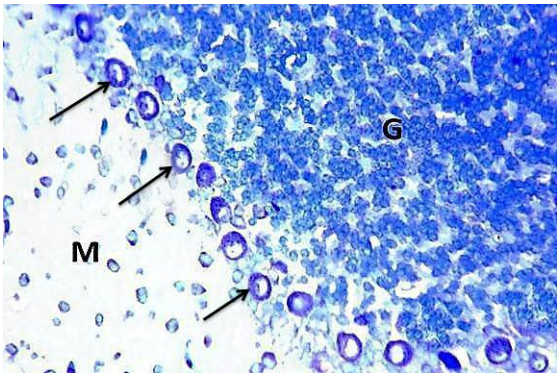


Fig (29): A photomicrograph of a section of the cerebellar cortex of a control rat aged 28 days showing the dark blue colour of staining for Nissl's granules in the molecular cell layer (M), the Purkinje cells (arrows) and in the granular cells (G). **(Toluidine blue x 400).**

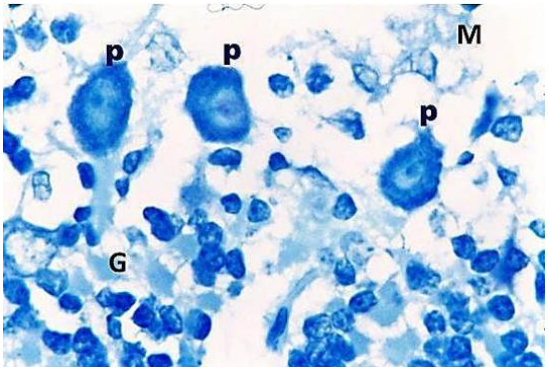


Fig (30): A photomicrograph of a section of the cerebellar cortex of a control rat aged 28 days showing the molecular layer (M) and the rounded Purkinje cells (P) rounded each having a big vesicular nucleus with a prominent deeply stained nucleolus and surrounded by Nissl's granules. The granular cells (G) are numerous small closely packed cells, each has oval deeply stained nucleus and scanty cytoplasm. **(Toluidine blue X 1000, oil immersion).**

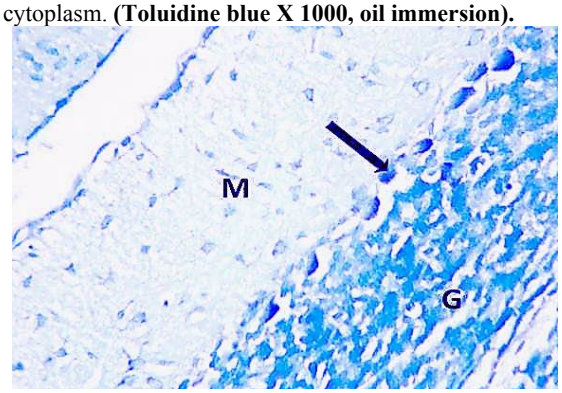


Fig (31): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 28 days showing decrease in the intensity (light blue) staining of the molecular cell layer (M), the Purkinje cells (arrow) and the granular layer (G). **(Toluidine blue x 400).**

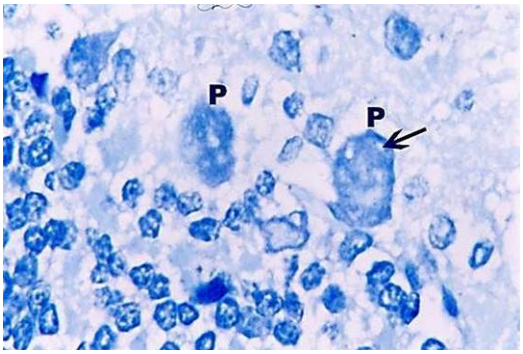


Fig (32): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 28 days showing ill defined and irregular Purkinje cells (arrow) with appearance of vaculations inside them. There is decrease in the intensity staining for Nissl's granules in all layers of the cerebellar cortex. **(Toluidine blue x 1000, oil immersion).**

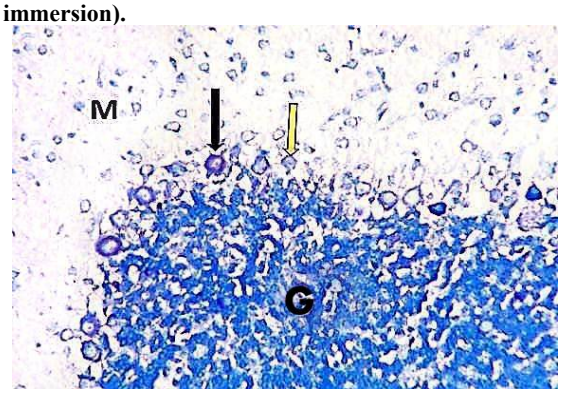


Fig (33): A photomicrograph of a section of the cerebellar cortex of a rat aged 28 days treated with colchicine + lithium chloride showing the molecular cell layer (M) is more or less as control group. There is improvement in the intensity staining for Nissl's granules (dark blue) in the Purkinje cells (black arrow). Few of them are degenerated & lightly stained (yellow arrow). The granular cells (G) are darkly stained. **(Toluidine blue x 400).**

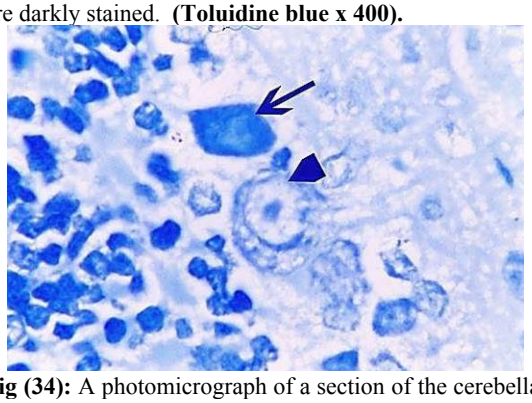


Fig (34): A photomicrograph of a section of the cerebellar cortex of a rat aged 28 days treated with colchicine + lithium chloride showing some Purkinje cell remain more or less like control with increased staining for nissl's granules & vesicular nucleus (arrow) and appear side by side with the lightly stained cell (arrow head).**(Toluidine blue x 1000, oil immersion**).

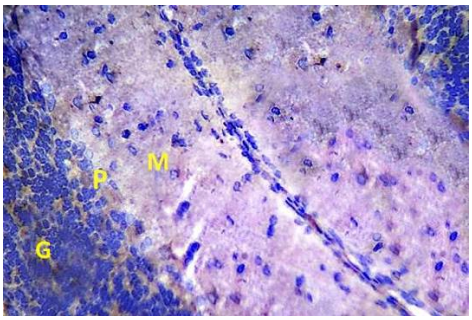


Fig (35): A photomicrograph of a section of the cerebellar cortex of control rat aged 28 days showing negative immune reaction to caspase-3 in the cells of cerebellar cortex. Negative reaction is indicated by very weak decrease in the brown staining of the cytoplasm of molecular layer (M), Purkinje cells (P) and granular layer (G). **(Immunoreactivity to caspase-3 x 400).**

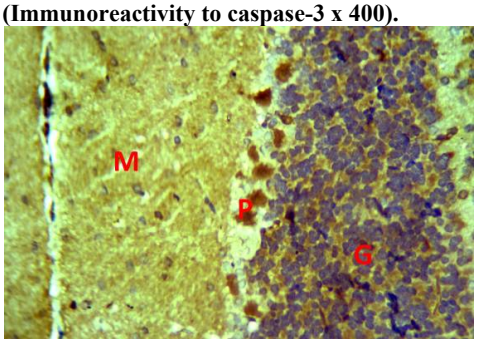


Fig (36): A photomicrograph of a section of the cerebellar cortex of a colchicine treated rat aged 28 days showing positive immune reaction to caspase-3 (dark brown staining) of molecular layer (M), Purkinje cells (P) and granular cells (G). **(Immunoreactivity to caspase-3 x 400).**

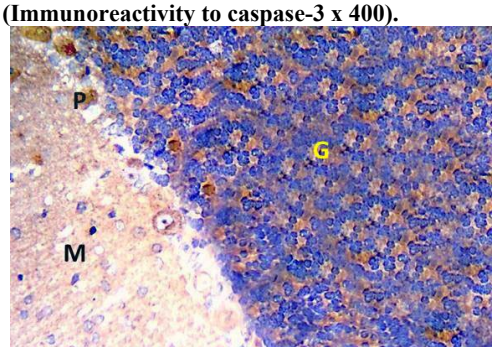


Fig (37): A photomicrograph of a section of the cerebellar cortex of a rat aged 28 days treated with colchicine + lithium chloride showing weak immune reaction to caspase-3 in the molecular cells (M), the cytoplasm of Purkinje cells (P) and in the granular cells (G) which is indicated by light staining of the cytoplasm as compared with the colchicine treated group. **(Immunoreactivity to caspase-3 x 400).**

***II- In vitro* studies** (Molecular detection of apoptosis by agarose gel electrophoresis):

A- In Control group:

There was no apoptotic bands could be detected in the lanes (1-5) representing tissue cultures of the control group as shown in (Fig. 38).

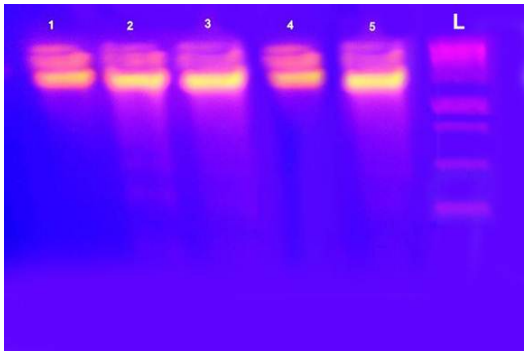


Fig (38): An electrophoretogram showing: five lanes of the control group of tissue cultures showing no fragmentation of DNA **(L → 200 bp ladder).**

Gel program analysis:

Table (5): The optical density of the control group of tissue cultures:

Base pair (bp)	*Lane 1 (Max OD)*	*Lane 2 (Max OD)*	*Lane 3 (Max OD)*	*Lane 4 (Max OD)*	*Lane 5 (Max OD)*
Intact DNA	*215.28*	*242.57*	*224.39*	*211.87*	*220.46*
800 bp	*19.229*	*107.18*	*99.356*	*23.446*	*102.665*
600 bp	*0.11220*	*52.829*	*52.741*	*0*	*49.07*
400 bp	*0*	*33.717*	*34.161*	*0*	*29.98*
200 bp	*0*	*3.9610*	*5.0537*	*0.142*	*3.456*

1. Molecular weight:

The graphs of the lanes of the control group showed only one peak of intact DNA and no peaks for released DNA fragmentation as shown in the following diagrams (1-5).

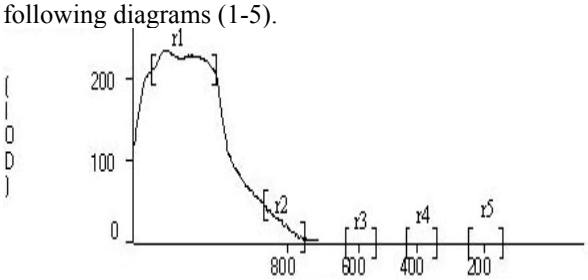


Diagram (1): Molecular weight in bp (lane 1).

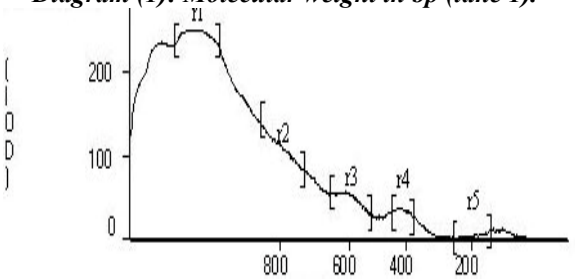


Diagram (2): Molecular weight in bp (lane 2).

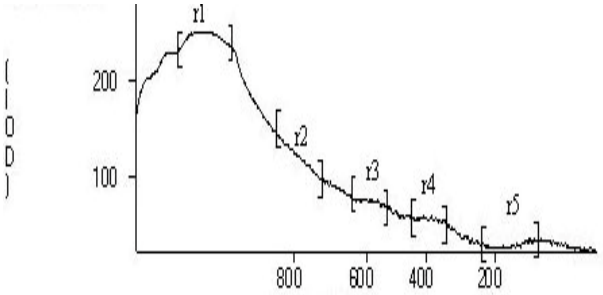


Diagram (3): Molecular weight in bp (lane 3).

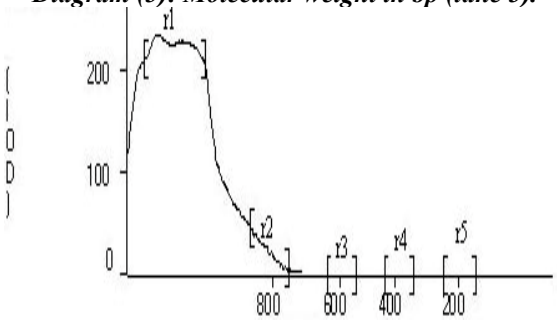


Diagram (4): Molecular weight in bp (lane 4).

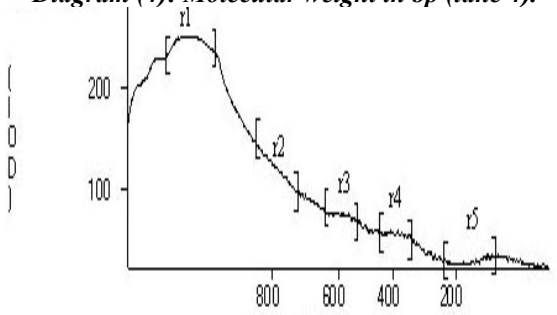


Diagram (5): Molecular weight in bp (lane 5).

B- In Colchicine treated group:

Apoptotic bands in the lanes representing tissue cultures treated with colchicine in the form of a ladder-like DNA fragmentation pattern (characteristic of apoptosis), were detected in lane (6-10) as shown in (Fig. 39).

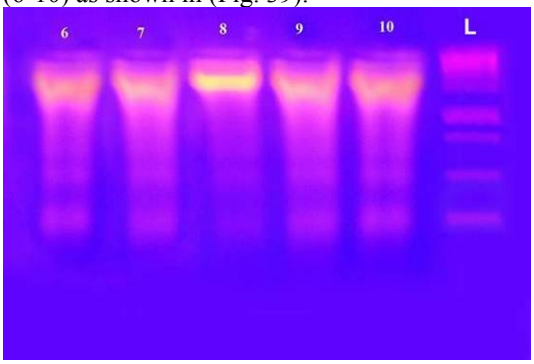


Fig (39): An electrophoretogram showing: five lanes of the colchicine treated group of tissue cultures showing severe fragmentation of DNA which is a characteristic of apoptosis **(L → 200 bp ladder).**

Gel- program analysis:

The optical density of the control group of tissue cultures. Table (6):

Base pair (bp)	*Lane 6 (Max OD)*	*Lane 7 (Max OD)*	*Lane 8 (Max OD)*	*Lane 9 (Max OD)*	*Lane 10 (Max OD)*
Intact DNA	205.38	206.66	204.36	206.93	203.65
800 bp	153.26	160.14	115.85	152.45	146.220
600 bp	116.16	116.02	81.917	110.63	108.45
400 bp	81.488	80.468	53.605	85.436	75.29
200 bp	66.556	80.566	49.634	75.56	58.028

1- **Molecular weight:**

The graphs of the lanes of the colchicine treated group showed peaks of intact and released DNA fragmentation. The peaks of released DNA showed molecular weight of approximately 200 bp and its multiplies which is a characteristic of apoptosis as shown in the following diagrams (6-10).

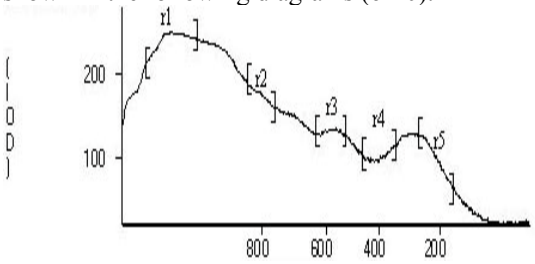


Diagram (6): Molecular weight in bp (lane 6).

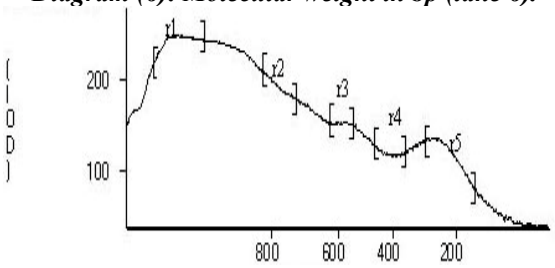


Diagram (7): Molecular weight in bp (lane 7).

Base pair (bp)	*Lane 11 (Max OD)*	*Lane 12 (Max OD)*	*Lane 13 (Max OD)*	*Lane 14 (Max OD)*	*Lane 15 (Max OD)*
Intact DNA	*214.95*	*219.557*	*210.597*	*217.78*	*211.009*
800 bp	*98.146*	*91.927*	*112.39*	*90.345*	*109.45*
600 bp	*51.898*	*54.020*	*77.156*	*55.659*	*71.076*
400 bp	*30.878*	*42.039*	*54.990*	*28.09*	*50.347*
200 bp	*13.493*	*26.137*	*68.805*	*10.890*	*66.132*

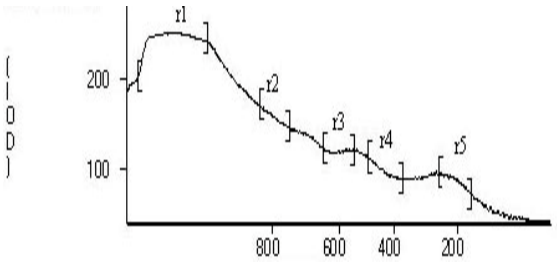


Diagram (8): Molecular weight in bp (lane 8).

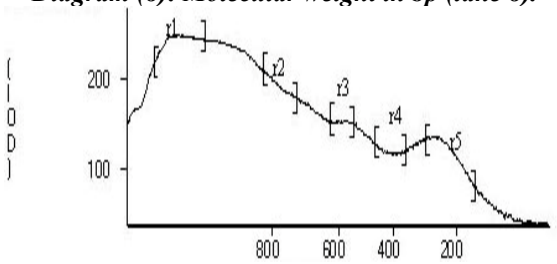


Diagram (9): Molecular weight in bp (lane 9).

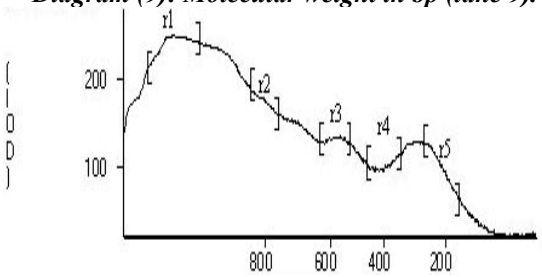


Diagram (10): Molecular weight in bp (lane 10).

C- In Protected groups:

There was decrease in the apoptotic bands in this group which could be detected in these lanes (11- 15), as compared with colchicine treated group (Fig. 40).

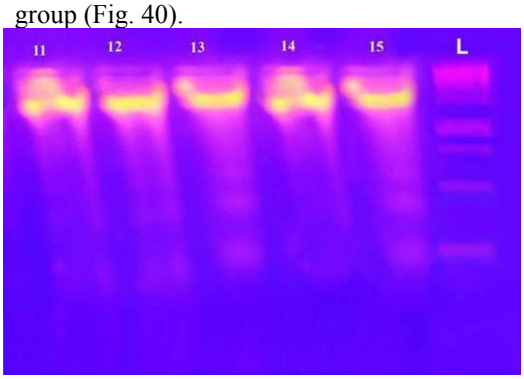


Fig (40):

An electrophoretogram showing: five lanes of the colchicine and lithium chloride treated group of tissue cultures showing slight fragmentation of DNA **(L → 200 bp ladder).**

Gel- program analysis:

1- **The optical density of the control group of tissue cultures. Table (7):**

2- **Molecular weight:**

The graphs of these lanes of (Colchicine + lithium chloride treated group) showed only one peak of intact DNA and less peaks for released DNA fragmentation than that of the colchicine treated group as shown in the following diagrams (11-15).

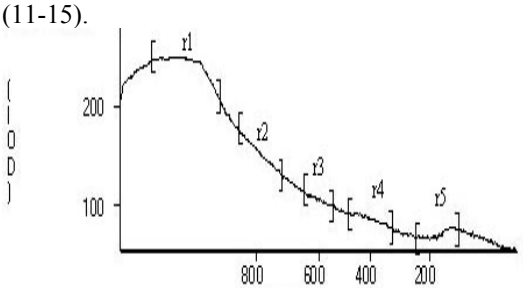


Diagram (11): Molecular weight in bp (lane 11).

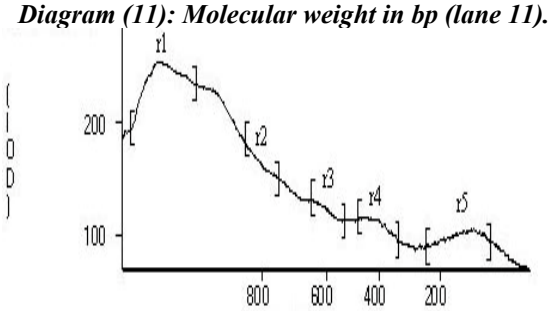


Diagram (12): Molecular weight in bp (lane 12).

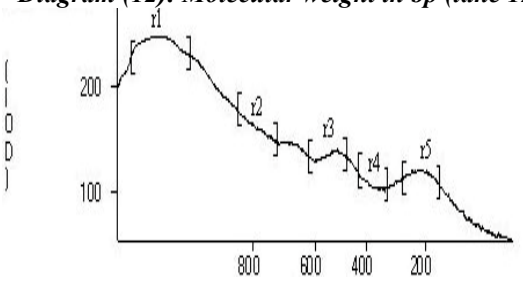


Diagram (13): Molecular weight in bp (lane 13).

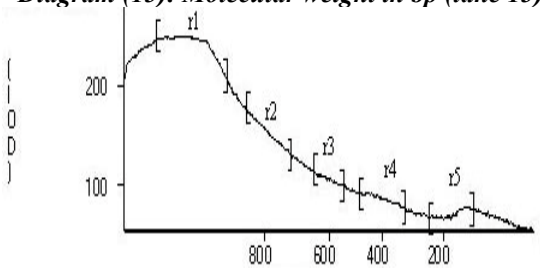


Diagram (14): Molecular weight in bp (lane 14).

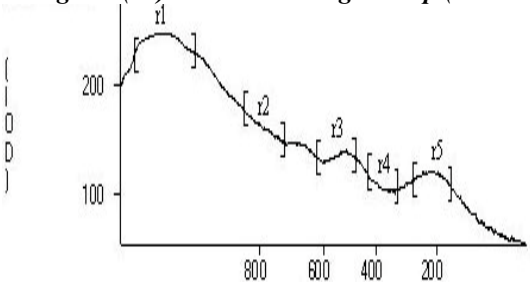


Diagram (15): Molecular weight in bp (lane 15).

4. Discussion

Colchicine is a naturally occurring alkaloid with weak anti-inflammatory activity derived from the autumn crocus Colchicum automnale and the glory lily Gloriosa superba. It has been used extensively in the treatment of gout for many centuries and also been recommended in preventing attacks of familial Mediterranean fever especially in children and in the treatment of primary biliary cirrhosis, amyloidosis, and condyloma acuminate and it is still widely used in the treatment of other several diseases **(Nabila, 2006).**

Colchicine is rapidly absorbed after oral administration, probably from the jejunum and ileum. It accumulates in kidney, liver, spleen, the gastrointestinal wall and leucocytes. Accumulation in these tissues may lead to toxicity **(El-Shafeey *et al.*, 2000).**

Colchicine has potent anti-mitotic activity, which is caused by its binding, both reversibly and selectively, to tubulin, the microtubular protein that disrupts the function of the mitotic spindles in those cells capable of dividing and migrating **(Wagenaar, 2004).**

Colchicine is a safe drug when used according to established therapeutic guide-lines but causes serious systemic effects if ingested in doses that exceed the recommendations **(Maxwell *et al.*, 2001).**

As far as side effects go, colchicine can cause a temporary reduction in the number of leukocytes (white blood cells) in the blood stream; afterward, the leukocyte count can rebound to abnormally high levels. It also causes teratogenic birth defects in laboratory animals, and so pregnant women with gout should not use colchicine-containing drugs **(Wagenaar, 2004).**

It is well known that colchicine triggers apoptosis in several neurons *in vitro* models such as organotypic hippocampal slice cultures **(Kristensen *et al.*, 2003)** or cerebellar granular neurons (CGNs) cultures **(Gorman *et al.*, 1999)**. Moreover, colchicine has been used as a model of acute microtubule (MT) disruption and has proved to be useful in testing neuroprotective drugs like flavopiridol and lithium **(Jorda *et al.*, 2004)**.

Colchicine causes apoptosis through the activation of intrinsic apoptotic pathway by the cytochrome C release and caspase-3 activation in CGNs ***(Gorman et al.*, 1999)** Colchicine enhances apoptosis induced by oxidative stress, which starts causing damage in the cellular membranes **(Canudas *et al.*, 2004)** and their effects can be reversed by inhibitors of cyclines such as flavopiridol or lithium **(Jorda *et al.*, 2003)**.

In agreement with previous studies**, Volbracht *et al.* (1999)** stated that, both cerebellar granular cultures (CGCs) in vitro and dentate granule cells (DGCs) in vivo underwent classical apoptosis mediated by caspase-3 activation upon colchicine exposure. The c-jun N-terminal kinase (JNK) pathway activation and phoshorylation/nuclear translocation of c-Jun preceded apoptosis in both CGC and DGC death.

The present study was carried out to investigate the histological, immunohistochemical and genetic changes that might occur in the cerebellum of albino rat offsprings as a result of exposure to colchicine and the possible protective role of lithium on it.

The albino rat was chosen for this work since it offered a number of advantages for experimental study; the white rat was cheep, easily obtained, housed and required minimal observation. Also, the albino rats had a relatively short period of pregnancy and their offspring were large enough to work conveniently.

In the present study, the cerebellum was selected because its postnatal development takes relatively long period **(Dobbing, 1968)** and undergoes more rapid growth and cell accumulation during the first three postnatal weeks **(Balazs *et al.*, 1971)**.

The present study demonstrated that, the external granular layer of 15 days old control rat's offsprings was formed of a sheet of closely packed cells of 4-5 cell layers. At the 28th day old, the external granular layer was not present. These observations were in agreement with **Obata and Fujita (1984)** who reported that, in the chick embryo cerebellar cortex, the external granular layer remained at its maximum width until 15 days and rapidly decreased thereafter.

The disappearance of the external granular layer was probably due to the migration of the cells from the external granular layer towards the molecular, purkinje and internal granular layers. This was in agreement with **El - shall (2004)** who reported that the external granular layer was the site of origin of the migrating cells which gave rise to many cellular elements of the cerebellar cortex.

The present study demonstrated that, the molecular layer at the 15 days old control rat offsprings appeared as a narrow zone beneath the external granular layer. However at the 28th day old control rat offisprings, the molecular layer was well developed and its thickness increased. These findings were in agreement with **Young and Heathi (2001)** who reported that the cerebellar cortex consisted of three layers. These layers are molecular, purkinje and granular cell layer.

In the present study, the Purkinje cells of 15 days control rat offsprings were arranged as a monocellular layer between the thin molecular layer and granular layer. At the 28th day posnatally, the Purkinje cells were flask shaped have big rounded nuclei, the tapering end of these flask- shaped cells and their cytoplasmic processes were directed towards the molecular layer while the broad bases were directed towards the internal granular layer. They were increased in size and became oval or flask- shaped with big rounded nuclei containing 1-2 nucleoli. These previous findings were in agreement with **Ralcewicz and Persaud (1995)** who reported that the Purkinje cells had a role in the induction of the development and maturation of granule cells. The identification of Purkinje cells were based on their size, flask shaped and direction of their dendrites.

This study demonstrated that, the internal granular layer showed wide spaces between the cells giving a loose appearance at 15 days old control rat offisprings. At older age the internal granular layer was increased in thickness and contained rounded and oval cells of variable sizes with deeply stained nuclei. These findings were in agreement with **Kamuro and Rakic (1998)** who mentioned that the migrating cells had different rates of movements. Cell movement was fastest in the molecular layer and slowest between the Purkinje cells. The cells of the internal granular layer resumed a rapid rate of migration until they reached the deep strata of the internal granular layer.

With exposure to colchicine either through in vivo or in vitro methods, it was found there were marked harmful toxic degenerative effects on the rat cerebellar cortex. This may be mediated by the activation of the apoptotic intrinsic pathway through cytochrome C release from mitochondria and the activation of caspase 3 **(Jorda *et al.*, 2004).** This was in agreement with ***Ben-Chetrit et al.* (2006)** who evaluated changes in gene expression induced by colchicine in an in vitro model of inflammatory disease.

Nabila (2006) stated that there were histological parameters which were followed in the spleen and testis of rats treated with double therapeutic doses of colchicine. The results obtained revealed histopathological changes in the spleen and testis. This may indicate the multisystem affection including cerebellar cortex as in our study.

Tsukidate *et al.* (1993) have suggested that the disruption of the integrity of microtubules by colchicine alters the permeability of the plasma membrane to extracellular calcium ions and induces an increased flux of these ions across the membrane of injured hepatocytes. A rise in the intracellular content of calcium activates an endonuclease with the resultant fragmentation of DNA. It has recently been reported that microtubule-interfering agents can suppress the closure of the permeability transition pore in tumor mitochondria, and that the membrane- bound tubulin or mitochondrial attachment to the microtubular network may be essential for the closing of this pore **(Evtodienko *et al.*, 1996)**; inhibition of the closing of the pore may permit inappropriate release of cytochrome C from the mitochondria **(Gorman *et al.*, 1999).** Disruption in cerebellar cortex structure noticed in our study may be explained by a similar mechanism.

Gorman *et al.* (1999) have demonstrated that colchicine induced apoptosis of cerebellar granular cells is accompanied by the early release of cytochrome C from the mitochondria into the cytoplasm, thus providing a possible mechanism whereby caspase-3, which is involved in the activation of DNAase, is activated in this neurotoxic process.

The results of the present study concerning the effects of the administration of colchicine on the development, and fate of the external granular layer, showed that the external granular layer was still easily differentiated up to the fifteenth postnatal day and also persisted up to the 28th day. The retardation of the disappearance of the external granular layer might be due to the reduction in the rate of migration of the external granular cells inwards towards the internal granular layer. These suggestions were in agreement with **Conradi (1987)** who explained the persistence of the external granular layer might be due to the failure of migration of the granular cell and the more possible delay in the rates of migration.

Eichenmuller *et al.* (2002) reported that there was an important gene induced by colchicine encodes EML1 (echinoderm microtubule associated protein like-1). This protein may modify the assembly dynamics of microtubules by shortening them due to lateral destabilization and thus affecting cytokinesis and cell migration. Therefore, the induction of this gene by colchicine may delay the migration of the granular cells.

In the present study, the molecular cell layer appeared as thin narrow zone beneath the external granular layer. At 15th day old rat, the molecular layer was less developed when compared with the control rat offsprings. At the 28th day old rat offsprings the molecular layer showed smaller thickness compared with control group. This was in agreement with **Altman (1972-a)** who suggested that the reduction in the thickness of the molecular layer was most probably due to delay in migration

of the granular cells and delayed ascending of the parallel fibers from these cells.

In the present work, the purkinje cell layer appeared small in size, less developed than the control one. At 15th day old rat offsprings, some of the Purkinje cells were arranged in one row, others haphazardly arranged in more than one row. The nuclei showed Pyknosis with condensation of chromatin. There was decrease in Nissl granules. At 28th day old rat offsprings, the Purkinje cells lost their characteristic feature and had ill defined shape with ill defined nuclei, there was focal loss of the Purkinje cells in some sections. Others showed pyknotic nucleus with vacuolated cytoplasm. In toluidine blue section, there was marked decrease in Nissl granules. These results were in agreement with **Vani and Reddy (2000)** who reported that the Purkinje cells in the cerebellum were the most affected cell population, and there was an increase in the number of granular cells but a decrease in the number of Purkinje cells and molecular cells.

Chen *et al.* (2002) found that the Purkinje cells of the cerebellum were affected due to accumulation of free radicals that led to reduction in the protein forming machinery of the neurons. This was in agreement with **Anamizu *et al.* (2006)** who stated that aging of the neurons leads to reduction in rRNA synthesis and ribosomes inside the cells resulting in impairment in protein synthesis inside the neurons. It was suggested that the observed reduction in ribosomes of the Prukinje cells was due to increase free radicals in these cells that disintegrate free and attached ribosomes.

In the present study, the internal granular cells were less densely packed together, some of them showed vacuolated cytoplasm.

Although in previous studies it has been evaluated changes in gene expression induced by colchicine in a in vitro model of inflammatory disease **(Ben-Chetrit *et al.*, 2006)**, there were no studies using neuronal cell cultures to analyze changes in gene expression mediated by colchicine. So the present study is an extension of our previous work on cerebellar granular neurons (CGNs) to increase our understanding of the potential pathways involved in colchicine-induced apoptosis in a neuronal cell culture. This might be explained by altered gene expression as mentioned above.

These data showed that the colchicine significantly activates the transcriptional activity of caspase 3 and 8 genes. This was in agreement with the reported activation of the classical apoptotic pathway on exposure to colchicine done by **Gorman *et al.* (1999).** This was also in agreement with our results either *in vivo* or *in vitro* studies considering caspase 3 activation and culture genetic studies.

In case of lithium chloride treated sections and granular neurons cultures treated with lithium chloride we found some protective effects to cerebellar cortical neurons by lithium chloride usage. This was in agreement with **Hennion *et al.*, 2002** who stated that the activation of caspase-3 by colchicine is inhibited in presence of lithium. Thus, this inhibition by lithium may explain the neuroprotective properties of this compound on neuronal cells. All these findings confirm earlier reports and are in line with evidence that lithium exerts wide neuroprotective and anti-apoptotic effects in a number of neuronal models.

Recommendations

From the results of the present work, it is advisable to avoid colchicine. If we use it, we should take the minimum dose of it or in company with lithium chloride. Colchicine should be avoided in the children, because their cerebellum was susceptible to be affected by colchicine.

So it is recommended that, this work needs for further extension(s) to include the CNS and the other body systems that may be affected by colchicine.

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