Effects of induced maternal hypothyroidism on the postnatal development of albino rat visual cortex and the ameliorative effect of Levothyroxine

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Abstract

Background: Central nervous system (CNS), in particular, depends on thyroid hormones (TH) for the growth and upkeep of normal physiological processes. The preferred medication for thyroid replacement therapy continues to be levothyroxine, a synthetic thyroid hormone.

Objectives: The goal of the current study was to determine whether maternally induced hypothyroidism could have an impact on the postnatal development of albino rat visual cortex and whether levothyroxine might have any protective effects or not.

Material and Methods: Twenty one (21) pregnant rats were randomly divided equally into three groups; control group (received distilled water orally daily from first day of gestation until day 20 after delivery), hypothyroid group (received Carbimazole orally in a dose of 5 mg/rat/ day from first day of gestation until day 20 after delivery) and hypothyroid group treated with levothyroixine (received Carbimazole orally in a dose of 5mg/ rat/ day for the same period concomitantly with Levothyroxine subcutaneously at a dose of 5µg/day/rat from day 10 of gestation until 20 day after delivery). Pups (newborn, 10 and 20 days) were anesthetized, sacrificed; their brains were processed for histological evaluation. Morphometric and statistical studies were done.

Results: Hypothyroidism induced visual cortex histological insults in the form of decreased cortical thickness and nuclear size and increase in packing of cells. Darkly stained cells were noticed. Clustering of pyramidal cells in ganglionic layer was not evident. Borders between layers couldn't be easily distinguished. These insults were ameliorated in hypothyroid rats treated with Levothyroxine.

Conclusion: Levothyroxine might protect against maternal hypothyroidism induced visual cortical neurotoxicity.

Key Words: Maternal hypothyroidism; Visual cortex; Levothyroxine.

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Introduction

The primary visual cortex is the primary cortical region of the brain that receives, integrates, and processes visual information that is relayed from the retina. The processed information from the visual cortex is subsequently sent to other regions of the brain to be analyzed and utilized**(Gipson and Kalivas, 2016)**.

The primary visual cortex (area 17) of the rat constitutes one part of the typical granular cortex with a prominent layer 4**(Scala et al., 2019).** In Nissl-stained sections, area 17 appears striated due to a high density of cells in layers 4 and 6 and a low density of cells in layers 5 and 2/3**(Senzai and Buzsáki ,2019).**

Studies have shown that neurons destined for the cerebral cortex are produced in the ventricular zone (VZ) of the lateral ventricles which is formed of neuroepithelial cells close to the ventricular lining of the neural tube. Neurons generated early in the VZ migrate radially towards the surface of the cerebral vesicles to form the primordial plexiform layer or preplate. Neurons generated later migrate to form a layer within the preplate, the so-called cortical plate (CP), thus splitting it into a superficial marginal zone (MZ) (layer I) and a deep subplate (SP). The neurons of the CP assemble into layers II-VI in an 'inside-out' sequence: The earliest-formed neurons occupy the deepest cortical layers, and successively later-formed neurons migrate past those generated earlier to occupy progressively more superficial laminar positions**(Wullimann et al., 2011).**

There is an abundance of pyramidal and non-pyramidal (stellate) neurons that are functionally and morphologically heterogeneous in the rat visual cortex. About 80% of thepopulations are pyramidal cells. Although non-pyramidal neurons are present in all layers,they concentrate in layers 2/3 and 4**(Johnson et al., 1988).**The importance of thyroid hormone transfer from the mother to the fetus during the second half of human pregnancy has received increasing acceptance. Thyroid hormones are essential for the development and maintenance of normal physiological processes, especially those of the CNS. Evidence from rat researches suggests widespread effects of TH on neuronal proliferation, migration and differentiation. It also affects neurite outgrowth, synaptogenesis and myelination. There is also increasing awareness of the importance of maternal thyroxine for the brain development early in pregnancy when the mother is the only source of thyroid hormone for the fetus**(Lavado-Autric et al., 2003)**.

Carbimazole is an antithyroid drug. It is a prodrug converted to methimazole after administration. Methimazole undergoes transplacental passage and is transferred to breast milk**(Schellack, 2013).**

Levothyroxine (LT4) is a synthetic TH that remains the drug of choice for thyroid-replacement therapy. The aim of the treatment is to normalize increased TSH levels. Thyroxine from the mother crosses to the fetus through placenta and pass in mother's milk to the neonates **(Higuchi, et al., 2005).**

Due to scarcity of previous histological researches dealing with the effect of Carbimazole on the development of rat visual cortex, the present study was designed to detect theameliorative effect of maternal induced hypothyroidism on the postnatal development of the visual cortex of the albino rat and the possible protective role of Levothyroxine against maternal induced hypothyroidism.

Material and methods

Experimental animals

A total number of 21 (180-200 gm) adult female and 5 (200-250 gm) adult male albino rats were obtained from the Animal House, Faculty of Medicine, Assiut University. They were maintained at the animal house with 12:12 hr. light: dark cycle and were kept under controlled temperature. Food and water were available ad libitum.Each four dams were matted with a single male overnight, vaginal smears were taken and spread on a slide and stained by Shori stain. The estrus period was characterized by the presence of non- nucleated epithelial cells. After appearance of the mucous plug, the vaginal smear of pregnant rats was found to contain non-

nucleated epithelial cells, a large amount of mucous and leukocytes. Pregnant female rats were singly housed and randomly divided into 3 equal groups; each group contained 20 rats.

Experimental chemicals

Carbimazole is a common antithyroid drug found in the form of tablets. Each tablet contained five mg of the active principal. It is a product of Chemical Industries Development. To prepare a suspension of the drug, five tablets were gridded and suspended in 25 ml of distilled water. Thus each one ml of the suspension contained one mg of the active principle.Levothyroxine in the form of Eltroxin tablet which is a synthetic Levothyroxine similar to the hormone produced by the thyroid gland. It is primarily used to treat hypothyroidism. Each tablet of Levothyrxine contained 50 µg of the active principal. It is manufactured by Glaxosmithkline Pharmaceuticals Ltd. To prepare a suspension of the drug, one tablet was gridded and suspended in 10 ml 0.09 NaCl. Thus each 1 ml of the suspension contained fiveµg of the active principal.

Treatment schedule

Group I (Control group): Consisted of 20 rats and were given distilled water daily from the gestational day one until the postnatal day 21.

Group II (Hypothyroid group): Consisted of 20 rats and were given Carbimazole in order to induce hypothyroid state in a dose of 5mg/day/rat. The drug was administered through an intragastric intubation daily from the gestational day one until the postnatal day 21.

Group III (Hypothyroidism treated with levothyroxine): consisted of 20 rats and were given Carbimazole orally through an intragastric intubation daily from the gestational day one until the postnatal day 21 in a dose of 5 mg/day/pregnant rat, and Levothyroxine from gestational day 10 until postnatal day 21 in a dose of 5µg/day/rat in 1ml 0.09 NaCl subcutaneously.

According to **El-Bakry El-Gareib& Ahmed (2010),** the rats'offspring were obtained from all animals and were anaesthetizedby ether inhalation, then subjected to intracardiac perfusion of normal saline 0.9% NaCl in the left ventricle associated with opening of the right atrium and sacrificed at the following ages; newborn, 10 days and20days**(Shehata et al. 2017).**

Histological studies

The brains were extracted from the skulls and processed for light and electron microscopic studies.

A) Light microscopic study

Some brain specimens were fixed in Bouin's fluid and then prepared for light microscopic examination. Serial sagittal sections about 5µm in thickness were prepared and stained with gallocyanin – chromalum to study the cytoarchitecture of the visual cortex in new born, 10 and 20 days age groups.A total six rats in each age group were used. Steps were done according toBancroft and Gamble**(Bancroft and Gamble, 2008).**

B) Electron microscopic study

For electron microscopic study other brain specimens were fixed in gluteraldehyde and subjected to:

- Semi-thin sectioning (0.5-1 µm), staining with toluidine blue and examination by light microscopy; to identify tissue component such as nuclei, membranes and cytoplasm of the cells in 10 days' age groups.
- Ultrathin sectioning, staining with uranyl acetate and lead citrate and examination by Jeol- JEM-100 CXII electron microscopat the Eectron Microscopy Unit of Assiut University; to study the ultrastructure of layer IV of the visual cortex in 20 days' age group. Steps were done according to Bozzola and Russell**(Chandler and Roberson 2009).**A total six rats in the studied age group were used. The primary visual area was isolated by using a razor blade to make parasagittal cuts 3.5 and 2.5 mm from the midline. From each slab the cortex overlying the dorsal hippocampal commissure (about 3mm rostral to occipital pole) was cut out. The Ethical Committee of Assiut Faculty of Medicine approved the dealing of the animals.

Results

A. By light microscopic study using gallocyaninchrom alum stain, the following results were noticed:

Newly born albino rat

- **Control animals (group I):**

Gallocyanin stained sections of the visual cortex (**Fig.1.a&Fig. 2**) showed that the visual cortex had outer, middle and inner layers.The outer molecular layer was narrow and contained many Cajal cells. They were fusiform in shape and small in size with their long axes parallel to the pial surface. Few stellate cells were present. They were rounded with scanty cytoplasm.The middle cortical plate showed overcrowded cells with high affinity to stain in its superficial part. They were indistinct cells, small in size and rounded in shape with darkly stained nuclei. It would form layer II, III and IV in advanced age. In the deep part of the cortical plate the cells started to differentiate into pyramidal and non-pyramidal cells to form future layer V.The inner fusiform layer showed pleomorphism. It contained a large number of fusiform and granular cells of different shape and contour. It would be the future layer VI.

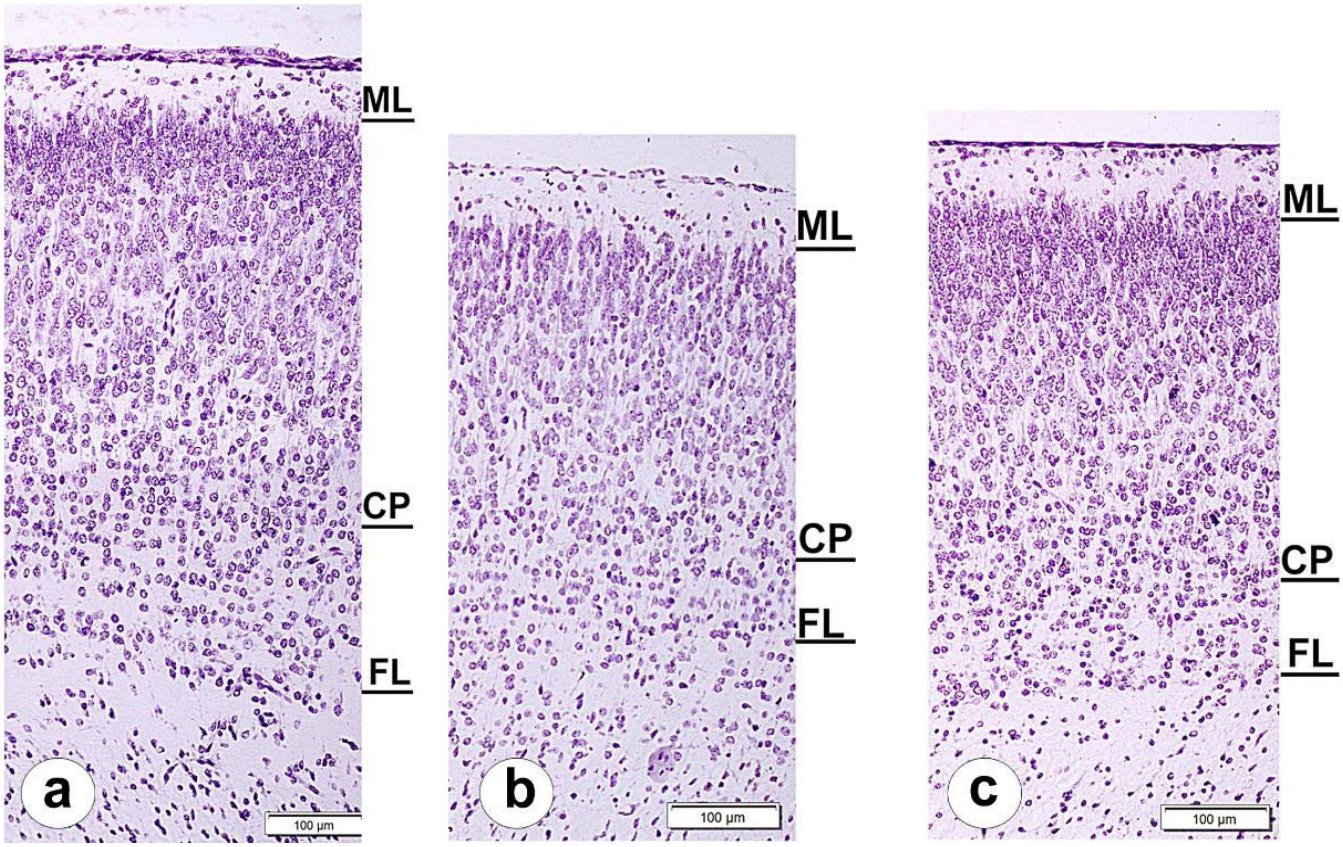

Fig.1. A photomicrograph of the visual cortex of new born rat. (**1a**):Control group showing trilaminar cortex; molecular layer (ML), cortical plate (CP) and fusiform layer (FL). (**1b**): Hypothyroid group showing trilaminar cortex; molecular layer (ML), cortical plate (CP) and fusiform layer (FL). Cortical thickness and cell size show apparent decrease. Cells appear to be more packed. (**1c**): Hypothyroid + Levothyroxine group showing trilaminar cortex; molecular layer (ML), cortical plate (CP) and fusiform layer (FL). Gallocyanin, X 200.

- **Hypothyroid animals (group II):**

The gallocyanin stained sections from the visual cortex of the hypothyroid group (**Fig.1.b&Fig.3**), showed the same pattern of the cortex, but the thickness of the cortex was apparently smaller than that of the control animals. The cells were smaller in diameter and more packed. Many darkly stained cells were present.

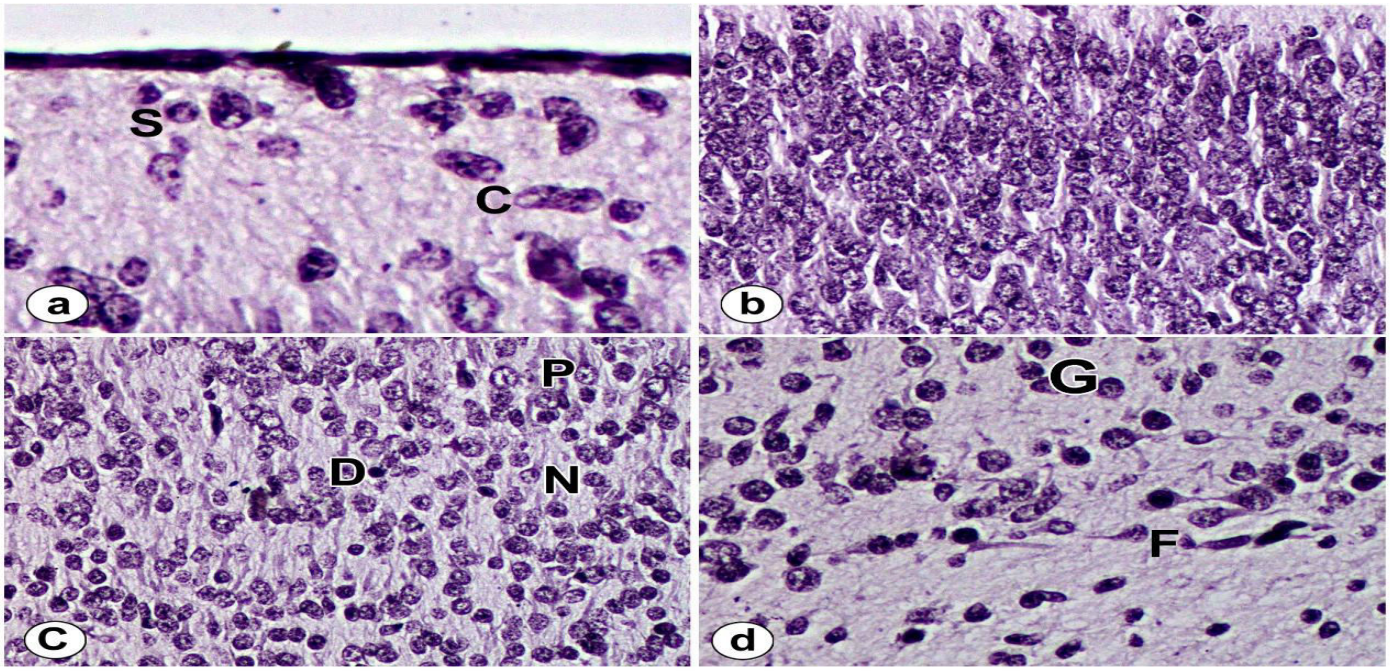

Fig.2. Magnified areas from figure 1.a showing; (**a**): Molecular layer with horizontal Cajal cells (C) and rounded stellate cells(S). (**b**): Superficial part of cortical plate having overcrowded rounded indistinct cells and small in size with high affinity to stain. (**c**): The cortical plate deep part showed that the cells started to differentiate into pyramidal (P) and non-pyramidal (N) cells and dark stained cell (D). (**d**): Fusiform layer showed large number of fusiform (F) and granular cells (G).Gallocyanin, X 400

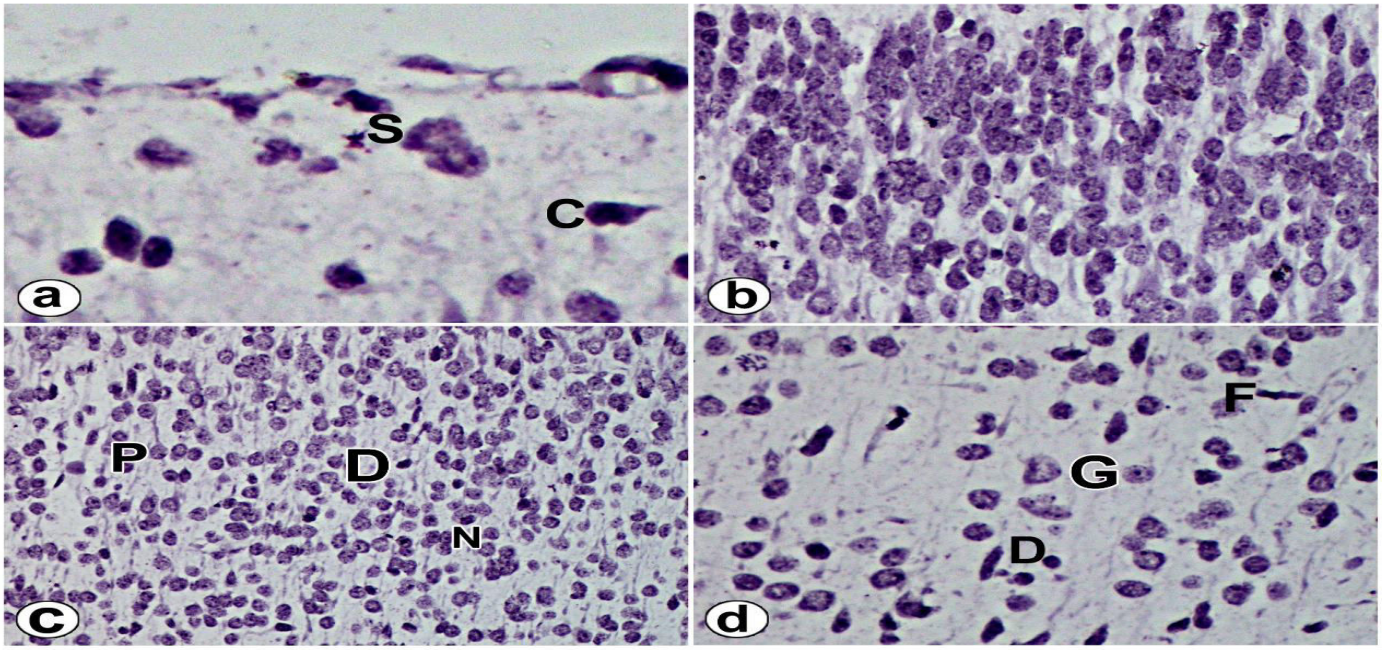

Fig.3. Magnified areas from figure 1.b showing; (**a**): Molecular layer with horizontal Cajal cells (C) and rounded stellate cells(S). (**b**): Superficial part of cortical plate containing overcrowded rounded indistinct cells and small in size with high affinity to stain. (**c**): The deep part of the cortical plate showed that the cells differentiate into pyramidal (P) and non-pyramidal (N) cells. There was large number of darkly stained cells (D). (**d**): Fusiform layer showed large number of fusiform (F) and granular cells (G), several darkly stained cells are observed (D). Gallocyanin, X 400

- **Hypothyroidism group treated with Levothyroxine (group III):**

The gallocyanin stained sections from the visual cortex of group III (**Fig.1.c&Fig.4**) showed normal appearance of the majority of cells. The cells had vesicular nuclei with prominent nucleoli. Few cells showed darkly stained nuclei.

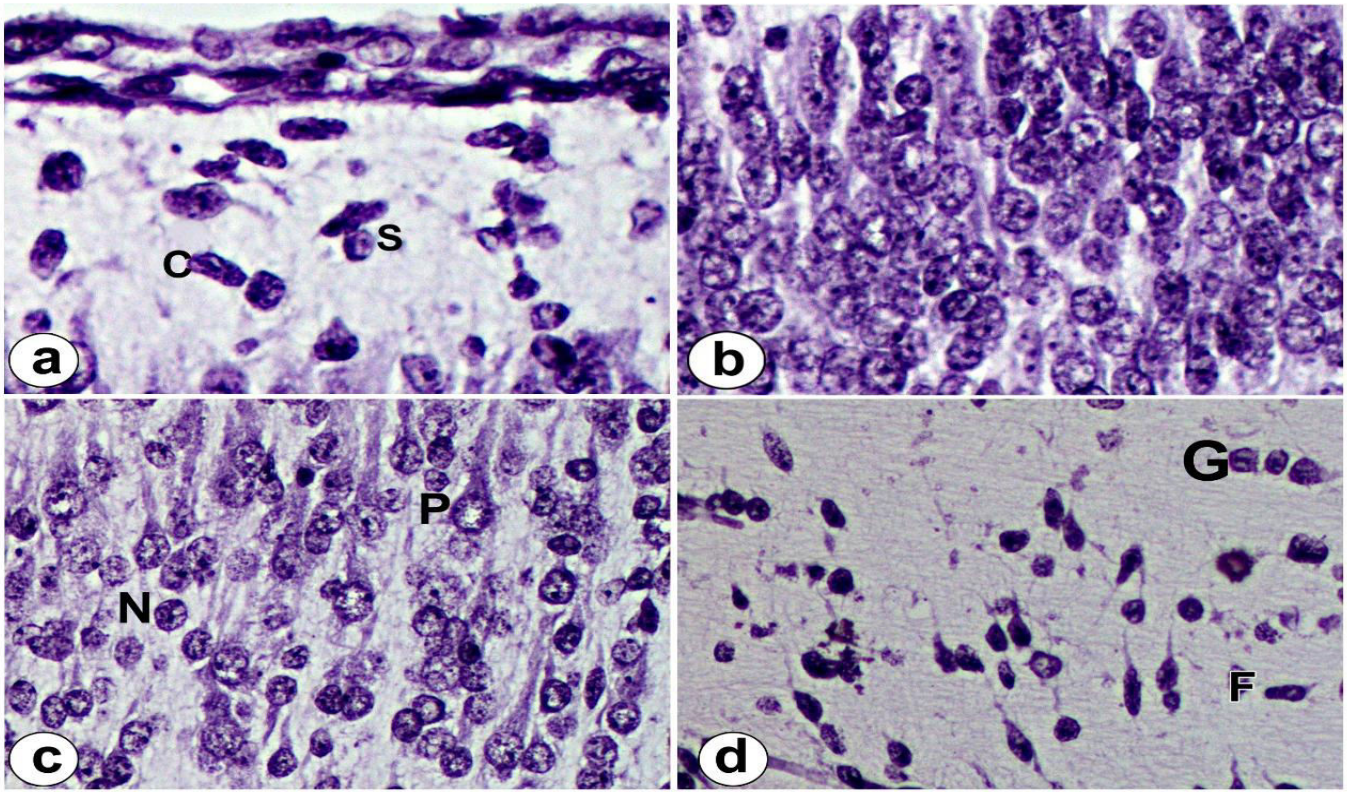

Fig.4. Magnified areas from figure 1.c showing; (**a**): Molecular layer with Cajal cells (C) and stellate cells(S). (**b**): Superficial part of cortical plate having overcrowded rounded indistinct cells small in size with high affinity to stain. (**c**): The deep part of the cortical plate shows that the cells differentiate into pyramidal (P) and non-pyramidal (N) cells. (d): Fusiform layer shows large number of fusiform (F) and granular cells (G). Gallocyanin, X400

Ten days old rat

- **Control animals (group I):**

The gallocyanin stained sections from the visual cortex (**Fig.5.a& Fig.6**) showed that the visual cortex had six layers.The molecular layer (I) increased in depth. There was an increase in the number of stellate cells while Cajal cells were reduced in number.Layers (II and III) couldn't be separated from each other. The outer part of these layers contained closely packed cells mainly non-pyramidal cells. The non-pyramidal cells were spherical in shape.In the deeper part, the cells were more widely spaced and there were small, intermediate and large sized pyramidal cells. The apices of the pyramidal cells were directed towards the pial surface. The internal granular layer (IV) had large pyramidal and non-pyramidal cells. The ganglionic layer (V) was distinct with large pyramidal cells which started to be arranged in clusters. There were also few non-pyramidal cells.The fusiform layer (VI) increased in thickness. The fusiform and granular cells increased in size and became more distinct.

- **Hypothyroid animals (group II):**

The gallocyanin stained sections from the visual cortex of the hypothyroid group (**Fig.5.b&Fig**.7) showed that the stratification was not clear except in layer I, V, VI.The thickness of the cortex was apparently less than that of the controls.The cells of all layers were more packed and clumsy than the cells of the control ones.The large pyramidal cells of layer V were more crowded, smaller in size and not arranged in clusters. Large number of darkly stained cells could be seen.

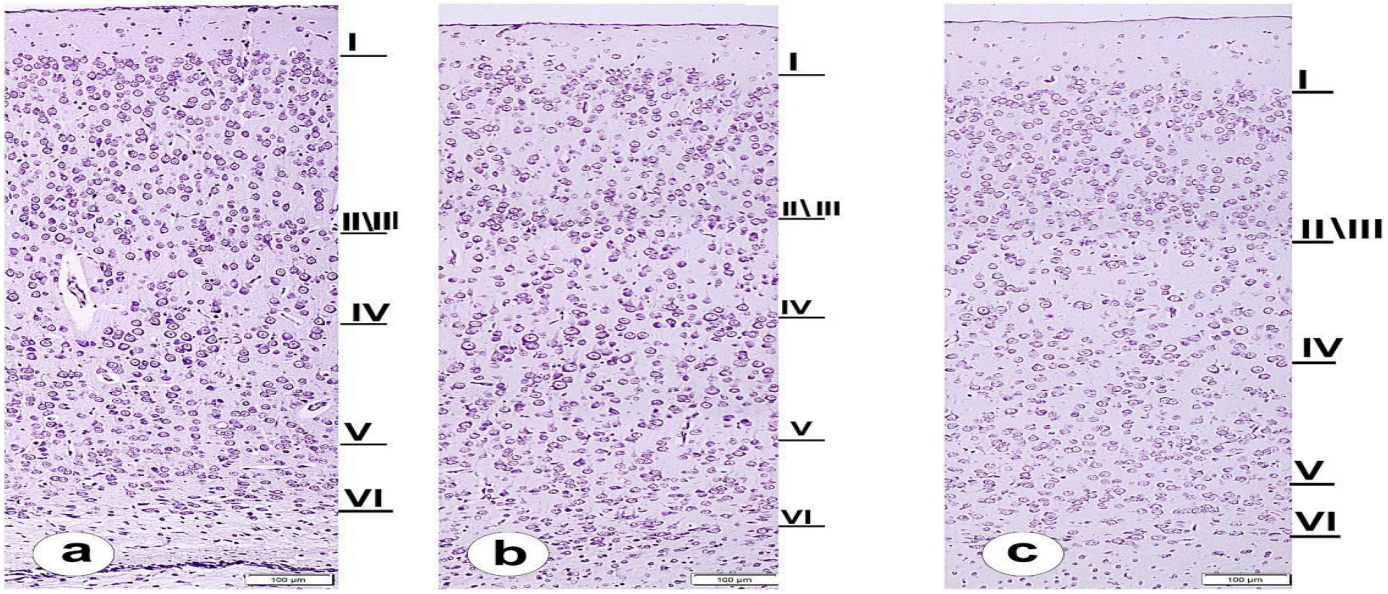

Fig. 5. A photomicrograph of the visual cortex of ten days old rat. (**5a**): Control group showing the six layers of the cortex. (**5b**): Hypothyroid group showing that the stratification is not clear. The thickness is apparently less than that of the controls. The cells appear to be more packed, smaller in size than those of the control. (**5c**): Hypothyroid + Levothyroxine group showing cortex has six layers. Gallocyanin, X 200

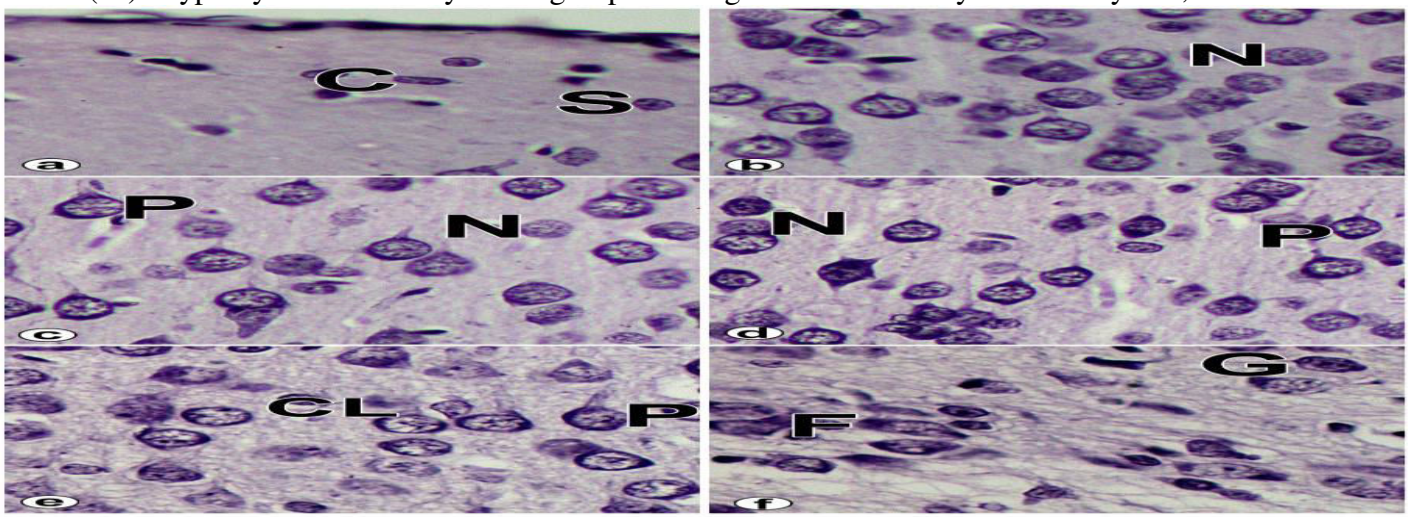

Fig.6. Magnified areas from figure 5a showing; (**a**): Molecular layer with horizontal Cajal cells (C) and rounded stellate cells(S). (**b**): Upper part of layer II\III containing mainly non pyramidal cells (N). (**c**): Lower part of layer II\III showing that the cells are more dispersed and differentiated mainly into pyramidal (P) cells. (**d**): Layer IV containing large pyramidal (P) and non-pyramidal cells (N). (**e**): layer V showing clusters (CL) of large pyramidal cells (P). (**f**): Fusiform layer showing large number of fusiform (F) and granular cells (G).Gallocyanin, X 400

- **Hypothyroidism group treated with Levothyroxine (group III):**

The Gallocyanin stained sections from the visual cortex of group III (**Fig.5.c&Fig.8**) showed normal appearance of the majority of cells, with vesicular nuclei and prominent nucleoli. Few cells showed darkly stained nuclei.

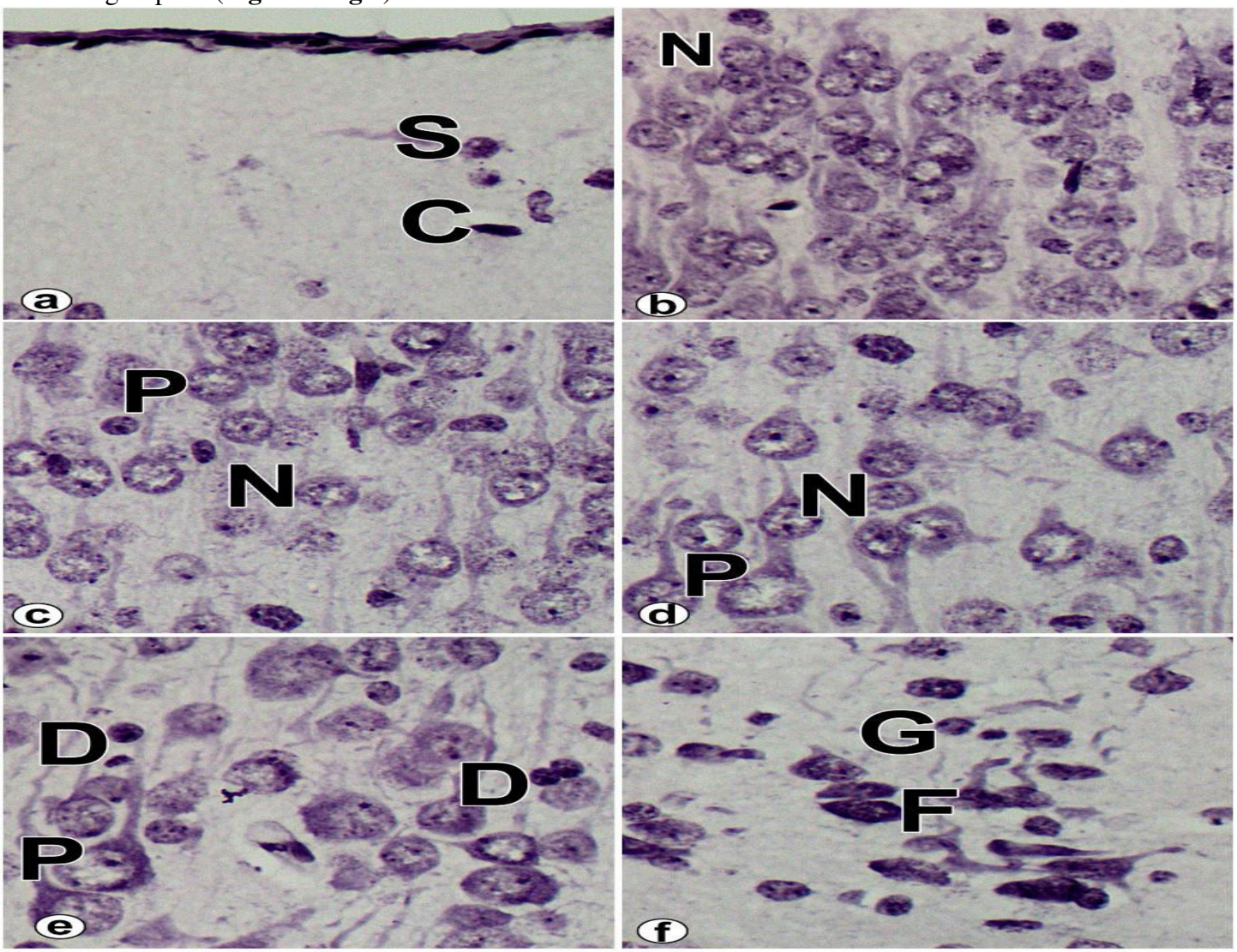

Fig.7. Magnified areas from figure 5b showing; (**a**): Molecular layer with horizontal Cajal cells (C) and rounded stellate cells(S). (**b**): Upper part and (**c**): Lower part of layer II\III showing more packed pyramidal (P) and non-pyramidal (N) cells. (**d**): Layer IV containing pyramidal (P) and non-pyramidal (N) cells. (**e**): Layer V showing packing of its large pyramidal cells (P) and loss of clustering. Large number of darkly stained cells (D) is noticed. (**f**): Fusiform layer showing large number of fusiform (F) and granular cells (G). Gallocyanin, X 400

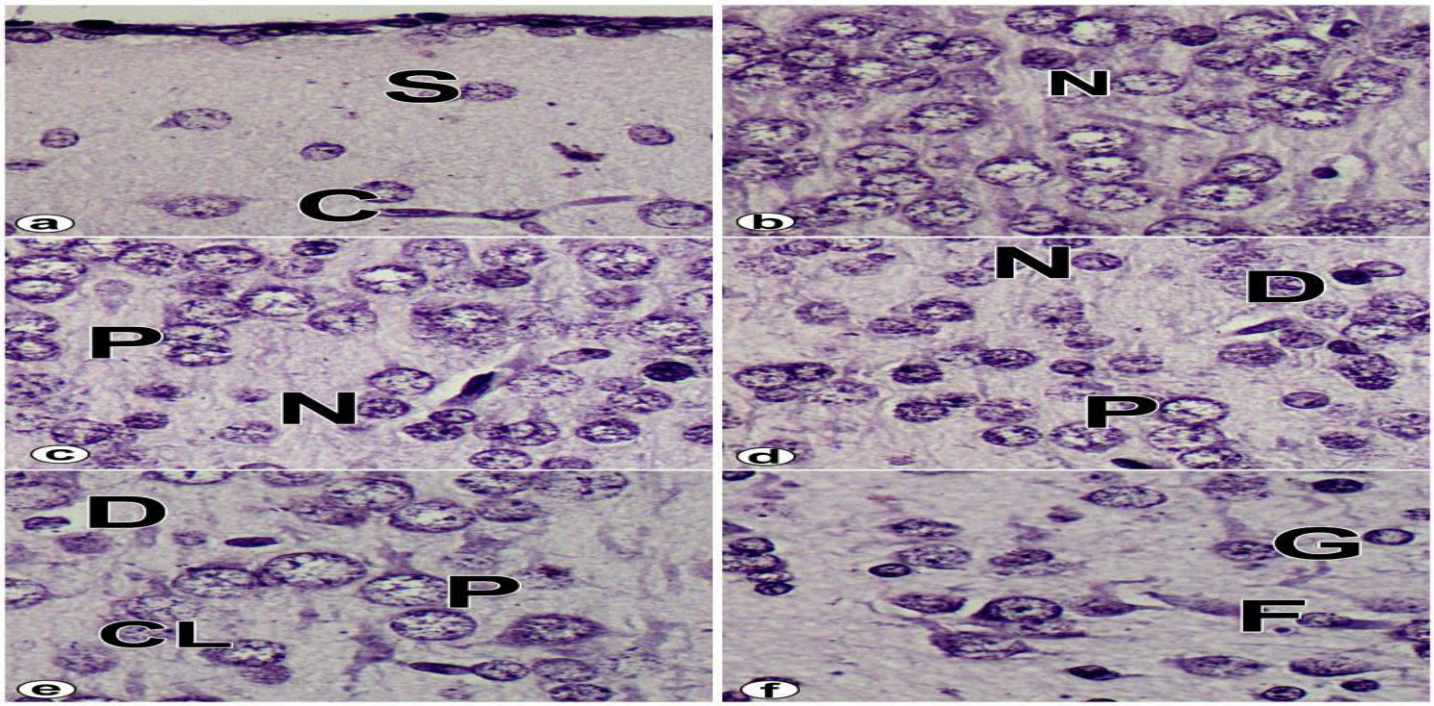

Fig. 8. Magnified areas from figure 5c showing; (**a**): Molecular layer with horizontal Cajal cells (C) and rounded stellate cells(S). (**b**): Upper part and (**c**): lower part of layer II\III showing pyramidal (P) and non-pyramidal (N) cells. (**d**): Layer IV containing large pyramidal cells (P) and non-pyramidal (N) cells. (**e**): Layer V showing large pyramidal cells (P) in clusters (CL). (**f**): Fusiform layer showing large number of fusiform (F) and granular cells (G). Most of the cells were similar tothose of the control group with vesicular nuclei and prominent nucleolus. Residual darkly stained cells are present (D).Gallocyanin, X 400.

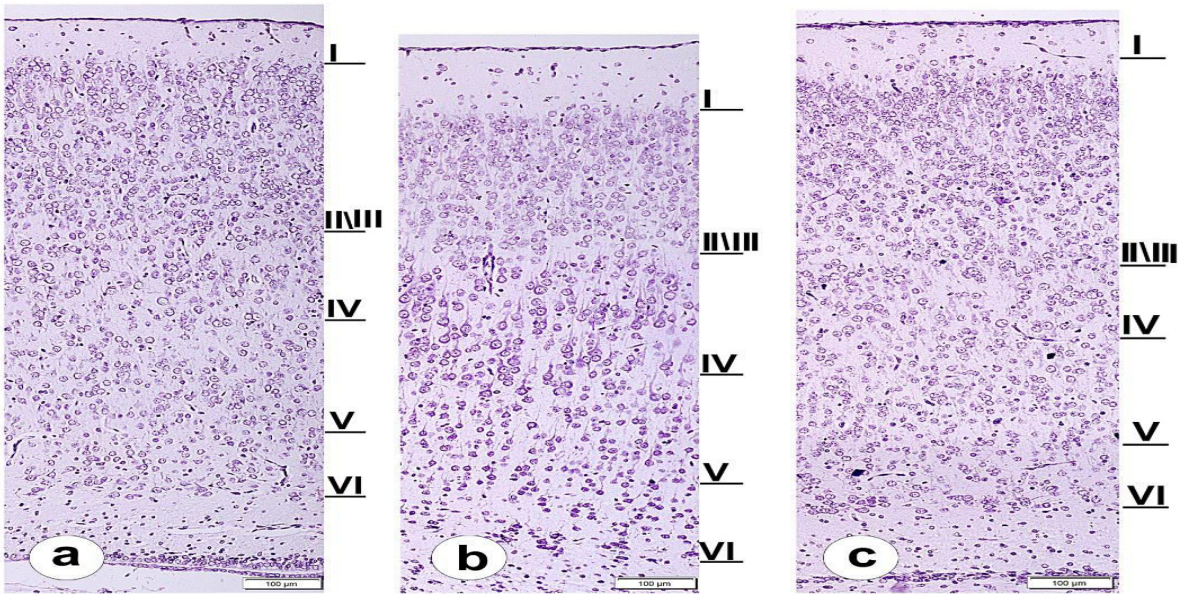

Fig.9. A photomicrograph of the visual cortex of twenty days old rat. (**9a**): Control group showing that the six layers of the cortex became more obvious and the cells appear larger in size and less packed than in the previous age. (**9b**): Hypothyroid group showing thatthe stratification is not clear. The thickness is apparently smaller than that of the control. The cells are more packed and smaller in size. (**9c**): Hypothyroid + Levothyroxine group showing that the cortex has six layers. Gallocyanin, X 200

Twenty days old rat

- **Control animals (group I):**

The gallocyanin stained sections from the visual cortexof the control group (**Fig.9.a & Fig.10**) showed that the stratification of the cortex became more obvious. The cells increased in size and became less packed. The molecular layer (I) became poorly cellular zone; contained few stellate and Cajal cells.Layer II/ III had more distinct cells than that of the previous age. The non-pyramidal cells in its outer part showed rounded nuclei and distinct nucleoli. There were also small, intermediate and large pyramidal cells mostly present in the inner part of this layer. The internal granular layer (IV) was identified by highly represented non-pyramidal cells with rounded nuclei, visible nuclear membrane and distinct nucleoli. The ganglionic layer (V) wasformed mainly of pyramidal cells. These cells arranged in clusters and produce vertical columns in between these clusters. There were also few non-pyramidal cells.The cells of the fusiform layer (VI) became more distinct and increased in size compared with the previous age. The upper part of layer VI contained smaller cells, closely packed, with rounded nuclei. The deep part contained horizontally elongated cells beside rounded neuronal nuclei.

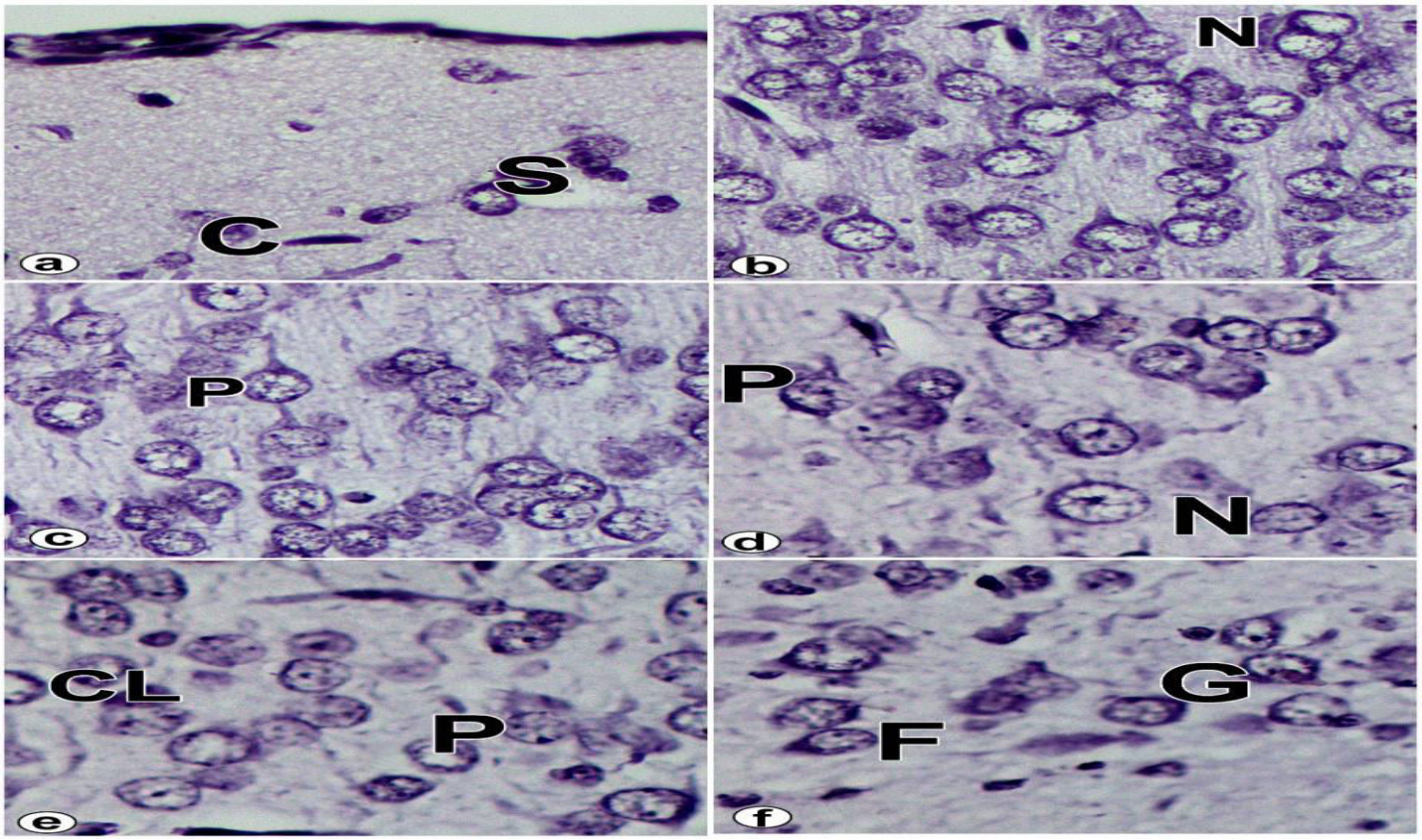

Fig. 10. Magnified areas from figure 9a showing; (**a**): Molecular layer with horizontal Cajal cells (C) and rounded stellate cells(S). (**b**): Upper part of layer II\III containing mainly non pyramidal cells (N) that showing a decrease in the cell packing than the previous age. (**c**): Lower part of layer II\III showing that the cells are more dispersed and differentiated mainly into pyramidal cells (P). (**d**): Layer IV showing large pyramidal (P) and non-pyramidal cells (N). (**e**): layer V shows clusters (CL) of large pyramidal cells (P). (**f**): Fusiform layer shows large number of fusiform (F) and granular cells (G). Gallocyanin, X 400

- **Hypothyroid animals (group II):**

The gallocyanin stained sections from the visual cortex of the hypothyroid group (**Fig.9.b&Fig.11**) showed that the stratification of the cortex was not clear as in the control animals and appeared to be smaller inthickness. The cells of the II/III and IV layers were more packed and the cells were apparently smaller in size.The large pyramidal cells of layer V were apparently smaller in size. The characteristic cluster pattern was not apparent. There were several darkly stained cells.

- **Hypothyroidism group treated with Levothyroxine (group III):**

The gallocyanin stained sections from the visual cortex of group III (**Fig.9.c&Fig.12**) showed normal appearance of the majority of cells. The cells had vesicular nuclei with prominent nucleoli. Few cells showed darkly stained nuclei.

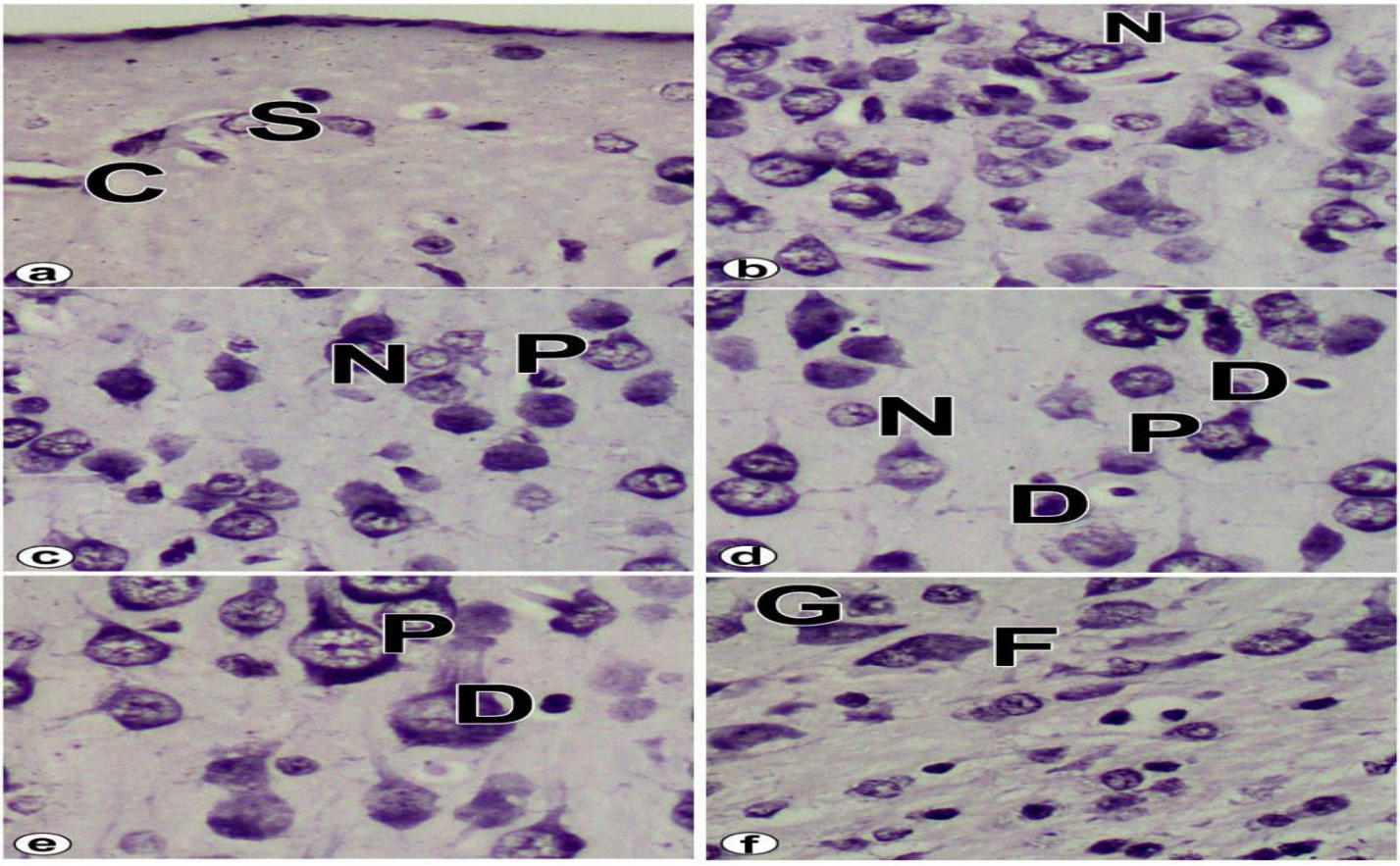

Fig. 11. Magnified areas from figure 9b showing; (**a**): Molecular layer with horizontal Cajal cells (C) and rounded stellate cells(S). (**b**): Upper part and (**c**): Lower part of layer II\III showing more packed pyramidal (P) and non-pyramidal (N) cells. Cells appears to be smaller and darker in color than those in the control group. (**d**): Layer IV contains pyramidal (P), non-pyramidal (N) cells and several darkly stained cells (D). (**e**): Layer V shows loss of clustering of its large pyramidal cells (P) and darkly stained cells (D). (**f**): Fusiform layer showing large number of fusiform (F) and granular cells (G). The cells appear smaller and more packed than those of the control group.Gallocyanin, X 400

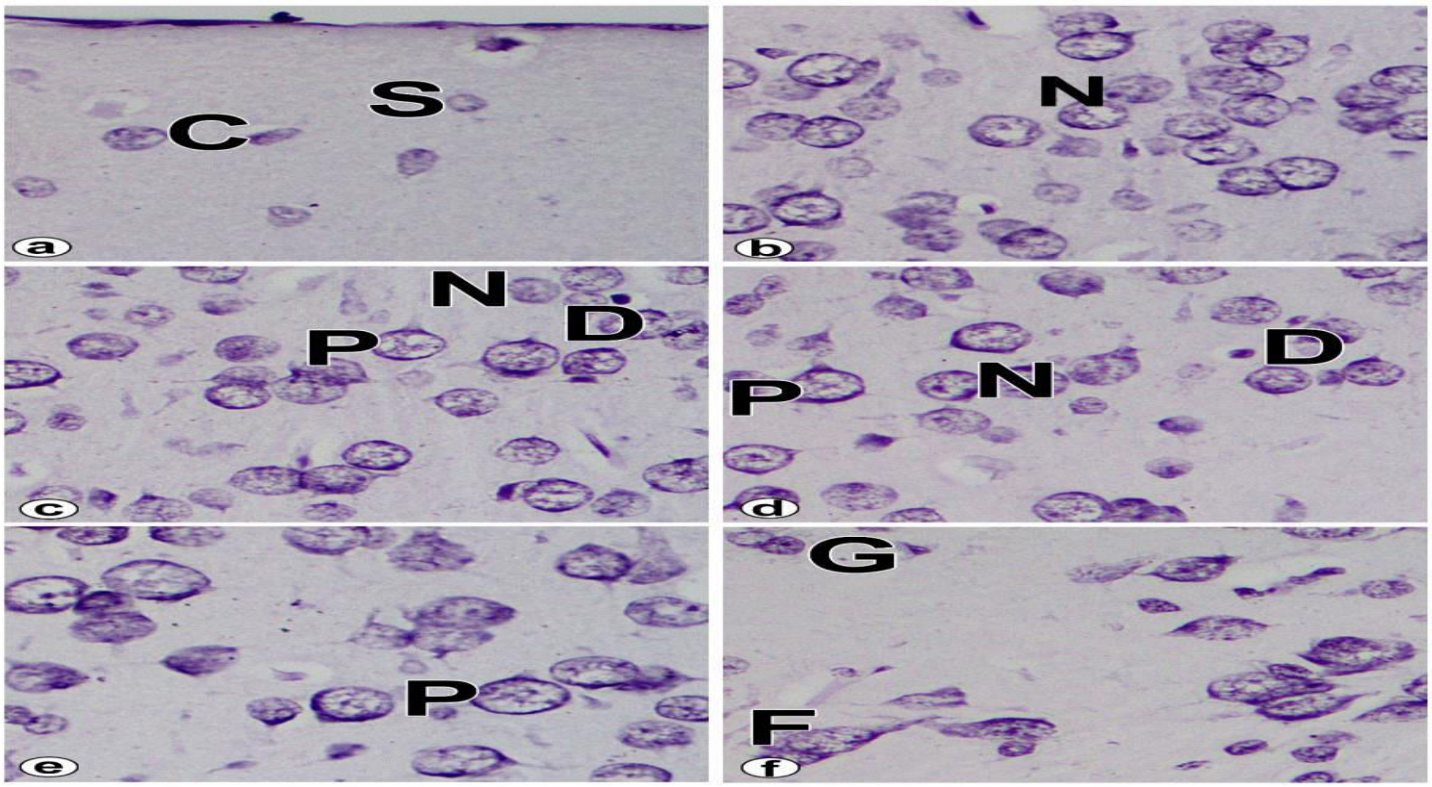

Fig. 12. Magnified areas from figure 9c showing; (**a**): Molecular layer with horizontal Cajal cells (C) and rounded stellate cells (S). (**b**): Upper part and (**c**): lower part of layer II\III showing pyramidal (P), non-pyramidal (N) cells and residual darkly stained cells (D). (**d**): Layer IV containing large pyramidal cells (P) and non-pyramidal (N) cells. Some darkly stained cells are present (D). (**e**): Layer V showing large pyramidal cells (P). (**f**): Fusiform layer showing large number of fusiform (F) and granular cells (G). Most of the cells have vesicular nuclei with prominent nucleoli. Gallocyanin, X 400

B. **By light microscopic studyusing Toluidine blue stain**, the following results were noticed:

Ten days old rat

- **Control animals (group I):**

Examination of a semi-thin section of visual cortex stained with toluidine blue (**Fig. 13.a**) showed that the pyramidal cells appeared triangular in shape. They had large oval shaped nuclei with prominent nucleoli and long apical dendrite. They were surrounded by a considerable amount of cytoplasm rich in Nissl granules. The granular cells appeared rounded in shape and showed large rounded vesicular nuclei with prominent nucleoli. Their nuclei had granular evenly distributed chromatin.

- **Hypothyroid animals (group II):**

Examination of a semi-thin section of visual cortex stained with toluidine blue (**Fig. 13.b**) showed that several pyramidal and granular cells appeared shrunken with darkly stained nuclei. Some cells had pyknotic nuclei. Many cells had vacuolated cytoplasm and darkly stained nuclei.

- **Hypothyroidism group treated with Levothyroxine (group III):**

Examination of a semi-thin section of visual cortex stained with toluidine blue (**Fig.13.c**) showed that the majority of pyramidal cells appeared normal. They had oval nuclei with prominent nucleoli and long apical dendrite. Some cells had darkly stained nuclei and vacuolated cytoplasm. Nearly most of the granule cells had normal appearance. They were rounded and showed large rounded vesicular nuclei with prominent nucleoli. Few cells only showed vacuoles in their cytoplasm and darkly stained nuclei.

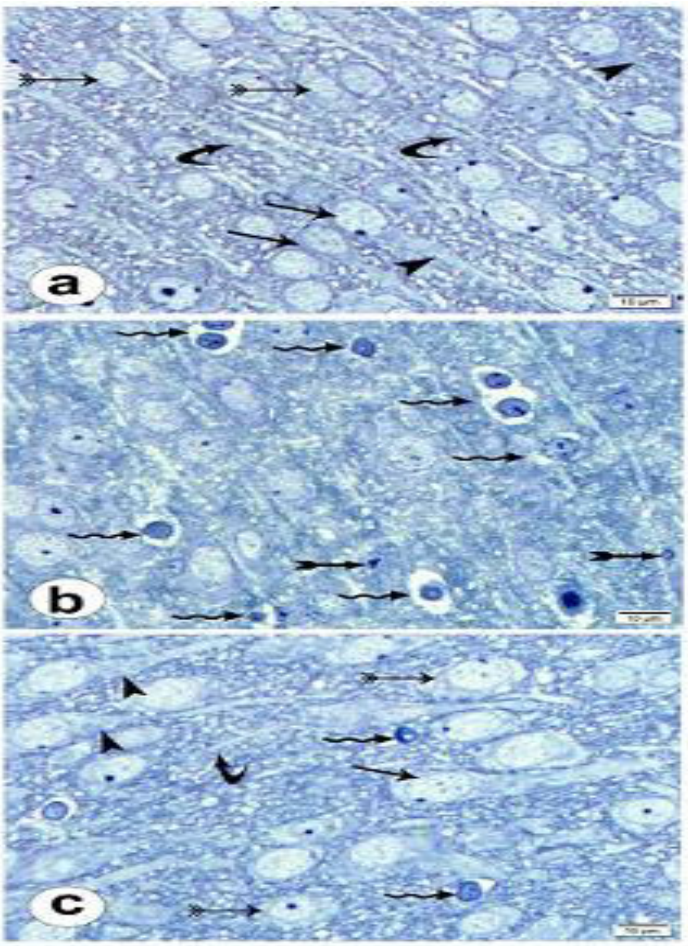

Fig. 13: A photomicrograph of a semithin section of the visual cortex of ten days rat. (**13a**): Control group showing that the pyramidal neurons (arrow) appear triangular in shape and contain oval nucleus and a prominent nucleolus and have long apical dendrite (arrow head). Granule cells (tailed arrow) contain large round nucleus and prominent nucleolus. Myelinated axons in the neuropil are seen (curved arrow). (**13b**): Hypothyroid group showing shrunken pyramidal and granular neurons with darkly stained nuclei and vacuolated cytoplasm (wavy arrow). Some cells have pyknotic nuclei (tailed arrow). (**13c**): Hypothyroid + levothyroxine group showing that most of pyramidal neurons appear normal (arrow) with vesicular nucleus and a prominent nucleolus and have long apical dendrite (arrow head). Most of granule cells appear normal (tailed arrow) with round nucleus and prominent nucleolus. Myelinated axons in the neuropil (curved arrow) are noticed. Residual neurons with darkly stained nuclei and vacuolated cytoplasm (wavy arrow) also present.Toluidine blue, X 1000

C. By electron microscopic study

Twenty days old rat

- **Control animals (group I):**

The nucleus of the pyramidal cells was oval in shape and had smooth contour. The nucleoplasm showed evenly distributed chromatin and the nucleoli were prominent. Thecytoplasm showed rough endopasmicreticulum (rER) aligned in parallel cisternae. It also contained polyribosomes attached to the cisternae of rER and free ribosomes scattered in the cytoplasm (**Fig. 14.a**). The nucleus of the granular neuron appeared to be spherical. The chromatin was evenly dispersed; prominent nucleolus was observed. The nuclear membrane appeared smooth and regular. The cytoplasm was rich in rER studded with polyribosomes, and between which laid many free ribosomes and mitochondria (**Fig. 15.a**).

- **Hypothyroid animals (group II):**

The pyramidal cells were apparently shrunken, Condensation of the nuclear chromatin, irregularity and indentation of the nuclear membrane were observed. The cytoplasm showed damaged mitochondria, marked loss of free ribosomes and dilated cisternae of rER (**Fig.14.b**).The granular cells were also shrunken and showed condensation of the nuclear chromatin. The cytoplasm contained many dilated cisternae of rER, scanty mitochondria and many vacuoles (**Fig. 15.b**).

- **Hypothyroidism group treated with Levothyroxine (group III):**

Examination of the pyramidal cells showed that their nuclei had evenly distributed nuclear chromatin. The cytoplasm showed mitochondria, free ribosomes and residual dilated rER cisternae. The nuclear membrane was smooth (**Fig.14.c**).Examination of the granular cells showed that the nucleus had evenly distributed chromatin. The cytoplasm had numerous mitochondria and free ribosomes as well as dilated rERcisternae. The nuclear membrane was smooth without irregularity (**Fig.15.c**).

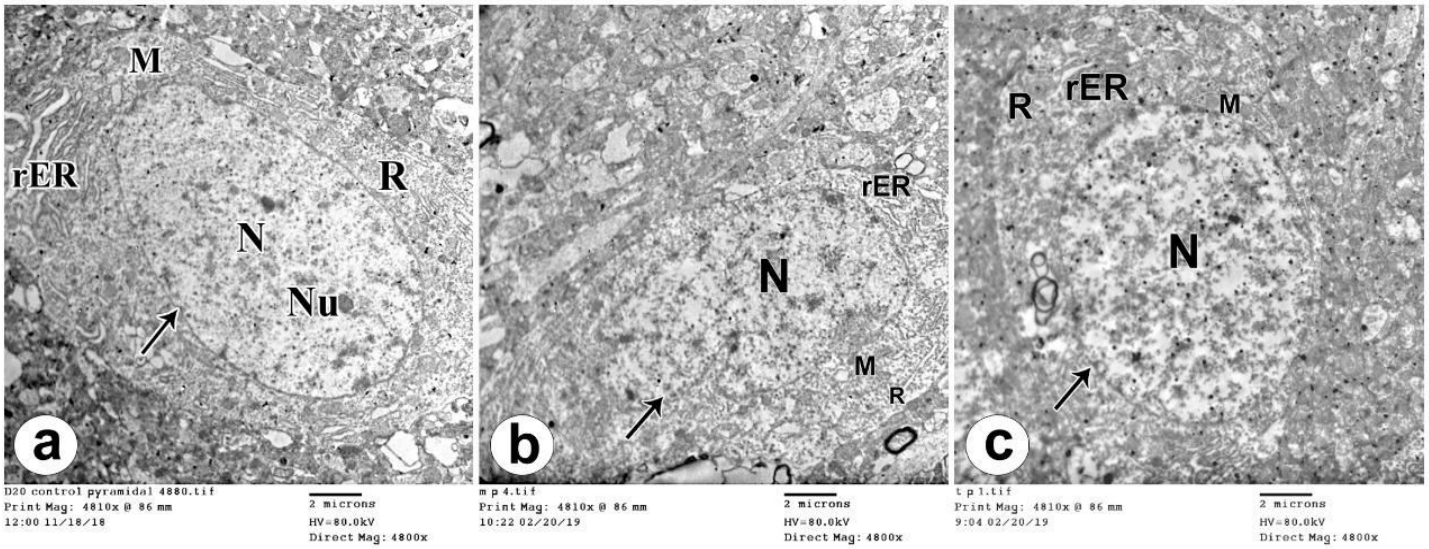

Fig. 14: An electron photomicrograph of the pyramidal cell of twenty days rat. (**14a**): Control group showing oval nucleus (N). It contains evenly distributed chromatin and prominent nucleolus (Nu). The nuclear membrane is smooth (arrow). The cytoplasm contains rough endoplasmic reticulum (rER), mitochondria (M) and free ribosome (R). (**14b**): Hypothyroid group showing that the cell appears shrunken. The nucleus (N) shows condensed chromatin and irregular nuclear membrane (arrow). The cytoplasm contains dilated rough endoplasmic reticulum (rER), destructed mitochondria (M) and scanty ribosomes (R). (**14c**): Hypothyroid + Levothyroxine group showing oval nucleus (N)containing evenly distributed chromatin with smooth regular nuclear membrane (arrow). It also contains mitochondria (M) and free ribosomes (R). Some rough endoplasmic reticulum (rER) cisternae are slightly dilated. TEM, X 4800

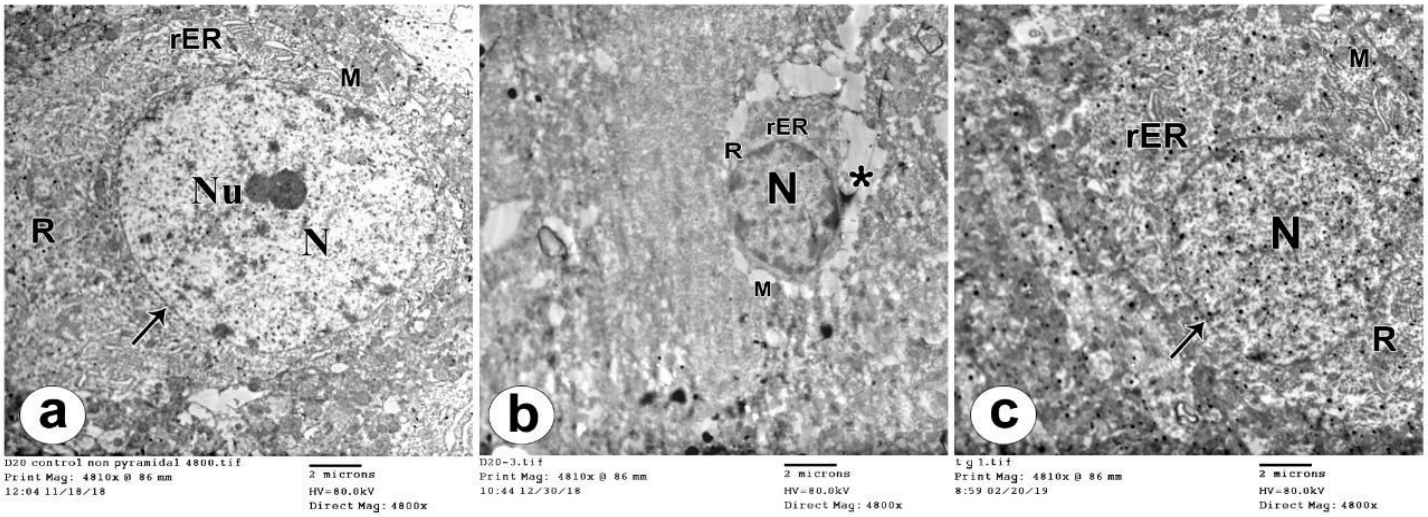

Fig. 15: An electron photomicrograph of the granular cell of twenty days rat. (**15a**): Control group showing rounded nucleus (N). It contains evenly distributed chromatin and prominent nucleolus (Nu). The nuclear membrane is regular (arrow). The cytoplasm contains rough endoplasmic reticulum (rER), mitochondria (M) and free ribosome (R). (**15b**): Hypothyroid group showing that the cell appears shrunken. The nucleus (N) shows condensed chromatin. The cytoplasm contains many vacuoles (star). It also contains scanty mitochondria (M) and dilated rough endoplasmic reticulum (rER). (**15c**): Hypothyroid + Levothyroxine group showing that the nucleus (N) shows evenly distributed chromatin. The nuclear membrane is regular (arrow). The cytoplasm contains rough endoplasmic reticulum (rER) some cisternae is slightly dilated, mitochondria (M) and free ribosome (R).TEM, X4800

Discussion

Developmental hypothyroidism was studied because it is one of the most common preventable causes of mental retardation**(Agrawal et al. 2015)** and may lead to irreversible morphological and cytoarchitectural abnormalities in the developing brain **(Ahmed et al. 2008).**This model reflects the situation of hypothyroid mother bearing a child with congenital hypothyroidism left without treatment after birth or that of myxoedematos cretin born in an area with severe iodine deficiency. The physiological role of thyroid hormone is to accelerate changes of gene expression that take place during development **(Bernal 2002).** This action is important for migration **(Bhanja and Jena 2013),**differentiation and neuronal outgrowth.

Migration take place before the onset of fetal thyroid hormone secretion and therefore, it is only dependent on maternal thyroid function. Cerebral cortex was studied because it is one of the most sensitive brain regions to perinatal hypothyroidism. Despite the differences in timing of neurodevelopmental events between human and rat; similarities between both species is that neocorticogenesis starts before the onset of fetal thyroid gland secretion **(Shibutani et al. 2009 andBerbel et al. 2010).**

The present study demonstrated that there is a gradual decrease in the Cajal cells and an increase in the non-pyramidal cells within the molecular layerin the control group. This may be explained on basis that Cajal cells disappear during cortical development. Another possibility is that they are transformed into other types of non-pyramidal cells**(Peters and Sethares 2002).**

The present work showed that there is gradual decrease in the packing of cells with age. This may be attributed to the corresponding growth of the tissue surrounding the cell body. The present study demonstrated that the clustering of large pyramidal cells in the ganglionic layer was evident by the 10^{th} postnatal day. These clusters may represent the cells that migrate into the visual cortex along the same radial glial fibers. Moreover, the dendritic bundles of the large pyramidal cells in the cluster might be related to the distribution of the thalamocortical afferents and serve as their main foci of termination**(Gabbott and Stewart 2012).**

By electron microscopic examination, the pyramidal cells in layer IV in control group at 20 days had oval nuclei with prominent nucleoli and evenly distributed chromatin. They were surrounded by regular intact nuclear membranes, the cytoplasm contained numerous rER, polyribosomes and mitochondria. Granular cell examination in the present work showed rounded nucleus with prominent nucleolus, evenly distributed chromatin and smooth regular nuclear membrane. Their cytoplasm contained rER, mitochondria and polyribosomes. These results are in accordance with other researchers**(Albrakati 2020).**

Examination of the hypothyroid group showed apparent decrease in the cortical thickness, the nuclear size and an increase in packing of cells. Multiple darkly stained cells were noticed. These results were supported by study of**El shaar et.al, (2013)**who studied the effect of hypothyroidism on hippocampus development. Thinning of the cortex of hypothyroid rats was explained by loss of neuronal population under maternal hypothyroidism, reduction in the rate of neurogenesis and the differentiation into neurons is compromised **(Mohan et al. 2012).**

At the age of 10 days, large pyramidal cells in layer V appeared smaller in size.The borders between layers couldn't be easily distinguished. These findings were supported by**Kumar et al. (2006).**It was suggested that TH promotes the survival of neurons as well as non-neuronal cells during development **(Barakat-Walter and Kraftsik 2018).**

In the present study, a loss of clustering of pyramidal cells in layer V in 10 day age group was observed. This may be attributed to shrinkage of diameter of neurons in the cluster making them more difficult to be identified compared with control group**(Gabbott and Stewart 2012)**.Examination of semi thin sections of hypothyroid group of 10 day showed that most of the neurons appeared shrunken with darkly stained pyknotic nuclei and vacuolated cytoplasm. This was in agreement with**(Tousson et al. 2012 andAl Jehani et al. 2017)**.

Electron microscopic examination of the pyramidal cells from hypothyroid group of 20 day revealed apparent shrinkage of cells, condensed chromatin, irregular and indented nuclear membrane and marked increase in density of cytoplasm with many vacuoles. Granular cell examination revealed shrunken nucleus that contained clumps of marginated chromatin. The cytoplasm contained scanty rER and mitochondria and showed vacuolations. These results were similar to those found by**Yang et al. (2015) andWang et al. (2017).**

Biochemical analysis suggested that neonatal hypothyroidism causes alterations in brain mitochondria. Mitochondrial damage leads to release of apoptosis inducing factors resulting in activation of caspase proteases that cause nuclear condensation and cytoplasmic fragmentation **(Omar 2016).**

In the present study, the treatment was started with levothyroxine on the 10^{th} day of gestation because this comes before the onset of neocorticogenesis and this is the optimal time for treatment of congenital hypothyroidism **(Prezioso &Chiarelli 2018)**.

The light microscopic examination of the Levothyroxine treated group showed normal appearance of the majority of cells with vesicular nuclei and prominent nucleoli. Few cells showed darky stained nuclei. These results were supported by**(Baghishani et al. 2018)**.Results of(**O'hare et al. 2015)**indicated that early T4 treatment has protective effect against the reduction of dendritic branching due to congenital hypothyroidism in hippocampus. This may explain the decrease in clumping than in the hypothyroid group that observed in the present study. Similar results were found after T4 supplementation to thyrodectomized adult rats**(Mohamed and Ahmed 2018).**

Electron microscopic examination of the pyramidal and granular cells in layer IV of visual cortex in the hypothyroid group treated with Levothyroxine at 20 day showed intact nuclear membranes. Nuclei had prominent nucleoli and evenly distributed chromatin. The cytoplasm showed mitochondria, ribosomes and residual dilated rERcisternae. These results were similar to that of **Wang et al. (2017).**The phenomenon that the brain injury was not completely healed after T4 supplementation might be related to the fact that when the serum level of TH returned to normal, the intracerebral hormone level was still insufficient **(Yang et al. 2015)**.

Conclusion

This work could explain the important role of TH on the processes that take place during fetal development such as cell proliferation and migration, that affect the cytoarchitecture of the visual cortex. Any situation resulting in a decreased availability of T4 to fetal brain is harmful for neurodevelopment. Early discovering of hypothyroidism in pregnant women and TH replacement therapy should be reconsidered to compensate for TH deficiency during pregnancy.

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