DOES ORAL CHLOROQUINE AFFECT THE HIPPOCAMPUS IN RATS? A CLUE TO CHLOROQUINE INDUCED NEUROPSYCHIATRIC ADVERSE EFFECTS

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ABSTRACT:

Background: *Chloroquine is widely used in medicine. The main indication for its usage is treatment of malaria. Reports about psychiatric side effects of chloroquine are rare. However, the list of recorded chloroquine induced neuropsychiatric disorders shows wide range of symptoms.*

Aim of the work: *In this study, the effect of oral chloroquine on the hippocampus of rats was assessed.*

Material and methods: *Twenty seven male adult albino rats were randomly divided into four groups. Group I (control group): nine rats were divided into I-a and I-b subgroups. Group II (chloroquine for two weeks): six rats received 4ml of distilled water solution/day containing chloroquine in a dose of 80 mg/kg bwvia oral gavage for two weeks. Group III (chloroquine for three weeks): six rats received chloroquine as in group II for three weeks. Group IV (chloroquine for four weeks): six rats received chloroquine as in group II for four weeks. After sacrifice, the hippocampi were retrieved, fixed, and processed for paraffin sections. H&E and Bielschowsky silver stains were applied and immunehistochemical staining for GFAP was performed to examine the distribution of astrocytes.*

Results: *Examination of different regions of the hippocampal formation revealed dark, shrunken cells, with pyknotic nuclei and pericellular spaces in all treated groups. Neurofibrillary tangles were also seen in some stained sections. Moreover, an increase in the density of astrocytes was also observed. Morphometrically, there was a decrease in the thickness of both pyramidal and granular layers of cornu ammonis and dentate gyrus respectively in all treated groups as compared with control group. All these changes appeared in group II, and were clearer in both groups III and IV.*

Conclusion: *It was concluded that oral administration of chloroquine caused duration dependent neuronal damage in the hippocampus of rats giving a possible explanation for chloroquine induced neuropsychiatric adverse effects.*

Key words: *Hippocampus, effect of oral chloroquine on hippocampus, dentate gyrus, cornu ammonis, GFAP, neuro-fibrillary tangles, rat.*

INTRODUCTION:

Chloroquine was synthesized in the 1930s by German scientists. It is a 4-amino-quinoline, and was named resochin[1].

Chloroquine is widely used in medicine. The main indication for chloroquine usage is treatment as well as prophylaxis of malaria. For prophylaxis, it is administered one week before reaching an endemic area and continued for one month after leaving it. In cases of chloroquine-resistant falciparum malaria, it is used in combination with proguanil[2].

Other indications are the treatment of rheumatoid arthritis and lupus erythe-matosus[2].

Despite a relatively few registered indications of chloroquine usage, there are other numerous indications, such as sarcoidosis, dermatomyositis, porfiriacuta-neatarda, and disseminated granuloma annulare[3].

Taking into consideration the wide range of chloroquine analogues usage, there are relatively few side effects when therapeutic doses are used. Common side effects include skin rashes, gastrointestinal disturbances, depigmentation, hair loss, visual disturbances, headaches, hypotension, convulsions, and extrapyramidal symptoms. In rare cases, hypersensitivity reactions such as angioedema and urticaria or bone marrow suppression can also occur [2].

Chloroquine has been found to cause retinopathy and corneal epithelial deposits[4]. The chloroquine induced retinal toxicity was found to be a serious adverse effect [5].

Chloroquine has serious cardiac adverse effects including cardiomyopathy and conduction abnormalities[6]. Refractory ventricular arrhythmia with QT prolongation was also recorded[7].

Reports about psychiatric side effects of chloroquine are rare. However, the list of recorded chloroquine induced neuro-psychiatric disorders shows wide range of symptoms[8].

Feeling sad, loss of interest, and suicidal ideas were reported [9].Moreover, over-activity, irritability, and talkativeness were also described [10]. Disorientation, confusion, agitation, and actual psychosis were also recorded[11&12].

Collins and McAllister[13] reported a case of female patient treated with chloroquine and complained of confusion, irritability, visual hallucinations, and paranoia with delusions.

Manic episodes with psychosis in the course of bipolar disorder were also reported[14].

More recently, derealisation, per-secutory delusions, anxiety, and visual illusions were found to be triggered by chloroquine administration [15].

In all these cases, chloroquine was prescribed for different indications. The onset of chloroquine-induced psychiatric side effects may vary greatly. It was reported that the latency between chloroquine usage and the onset of psychiatric disorders to range between 6 to 432 hours (M = 100.08 ± 96.00 hours)[16].

Moreover, all these psychiatric adverse effects of chloroquine were not dose-related. And there was no clear relationship between the severity of these effects and the dose of administered chloroquine [16]. So, during the differential diagnosis of chloroquine-induced psychosis, other common co-morbidities should be excluded, such as primary mental disorders, metabolic disorders, neuropsychiatric lupus, and glucocorticoid-induced psychotic disorder[8].

The hippocampus is a crucial component of human brain and brains of

other vertebrates. It is a part of the limbic system[17].

The hippocampal formation plays important roles in behavior inhibition, memory, and spacing [18-21].

Regarding to behavior inhibition, it was found that hippocampal damage leads to hyperactivity in animals, moreover, animals with hippocampal damage often have difficulty in inhibiting responses that they have been taught before, especially those responses that require being quiet [18].

Neuroscientists and psychologists have the same opinion about the role of hippocampus in memory. It plays a fundamental role in the setting up of new memories about practiced events as autobiographical or episodic memory[22].

Also, hippocampus plays a major role in navigation and spatial memory. Studies that have been conducted on freely moving rats revealed that many neurons in the hippocampus have "place fields". These fields send shots of action potentials when a rat passes through a certain part of the environment[23].

Different studies concluded the protective effects of chloroquine on the nervous system in certain conditions. Zhang et al.[24] have proved that chloroquine inhibited the functions and proliferation of glial cells in the hippocampus and cerebral cortex of rats, so, it could alleviate the seizure activities in case of drug induced seizures. Other studies have revealed that chloroquine may have an important therapeutic role in both acute and chronic neuropsychiatric disorders such as Alzheimer's disease (AD) and brain ischemia[25&26]. Then, the neuro-protective effects of chloroquine following traumatic brain injury were investigated in rat models. It was concluded that chloroquine attenuates autophagy and inflammation in rat hippocampus [27].

On the other hand, different studies have proved the neurotoxic effects of chloroquine on healthy tissues. The study of Adjene and Adenowo[28] has revealed that chronic administration of chloroquine caused cellular degenerative changes in the inferior colliculus in rats leading to adverse effect on the functions of the inferior colliculus. Also, prolonged duration of chloroquine administration led to retinopathy in rats [29, 30]. More recently, it was proved that chloroquine increased the oxidative stress as it might adversely affect the DNA in rat brain[31].

Despite the results of these studies, information about the effect of chloroquine on healthy brain tissue especially hippocampus remains rare. So, it was important to study the effect of chloroquine on the normal structure of the hippocampus in rats in a trial to find clue to the above mentioned neuropsychiatric side effects of this drug.

AIM OF THE WORK:

In this study, the effect of oral chloroquine on the hippocampus of rats was assessed.

MATERIAL AND METHODS:

Animals:

Twenty seven male adult albino rats (weighing 200 - 250 gm) were used. The rats were supplied water ad libitum, fed the standard rat chow, and kept under the same conditions throughout the duration of the study. The rats were obtained from the Ain Shams Faculty of Medicine Animal's House.

The study design:

The animals were randomly divided into four groups:

Group I (control group): Nine rats were further divided into two subgroups:

Group I-a: Three rats were left without any intervention and were sacrificed at the end of the experiment.

Group I-b: Six rats received 4 ml of distilled water every day via oral gavage. Every two rats were sacrificed after elapse of 2, 3, and 4 weeks.

Group II (chloroquine treated group for two weeks): Six rats received 4 ml of distilled water solution/day containing chloroquine in a dose of 80 mg/kg b.w[32] via oral gavage. The animals were sacrificed after two weeks.

Group III (chloroquine treated group for three weeks): Six rats received chloroquine as in group II and were sacrificed after three weeks.

Group IV (chloroquine treated group for four weeks): Six rats received chloroquine as in group II and were sacrificed after four weeks.

Chloroquine was purchased as Chloroquine 250 mg tablets (Alexandria Co. for Pharmaceuticals, Alexandria, Egypt).

Fixation and retrieval of the hippocampi:

Under anesthesia, a median incision was done in the chest of each rat, a needle was introduced into the left ventricle and the brain was fixed in situ by perfusion with warm formalin 10% in phosphate buffered saline (PBS). The brains were dissected rapidly from the skulls and kept immersed in the fixative for at least ten days. The hippocampi were obtained through parasagittal section in one hemisphere and a coronal section in the other hemisphere.

Histological study:

The brain specimens were prepared for paraffin blocks. Serial sections of the thickness of 5 µm were obtained and stained with Bielschowsky silver and H&E stains then examined by light microscope[33].

Immunohistochemistry:

In some paraffin sections, immune-histochemical staining for GFAP (anti-glial fibrillary acidic protein) antibody was performed to examine the distribution of the astrocytes[34]. The reaction appeared as brown cytoplasmic coloration.

Statistics and image analysis:

Six different fields from six different stained sections at a magnification x400 of six different rats in each group were examined for measuring the thickness of pyramidal layer of different areas of the *cornuammonis* and granular layer of the dentate gyrus. Distribution of astrocytes was also assessed on RGB stacks of the photomicrographs. A binary mask was overlapped on the areas of immune reactivity using threshold adjustment. All measurements were taken by the Image G software. The mean values as well as standard deviation (SD) were calculated using version 17 of SPSS program, IBM Corporation, New York, USA. One way ANOVA (analysis of variance) was performed, then post hoc test to compare between the studied groups. With regard to probability, a *P* value less than 0.05 was considered significant and those less than 0.001 were considered highly significant.

Ethical consideration:

All the experimental protocols were carried out according to the guidelines of the Committee of Animal Research Ethics (CARE), Ain Shams Faculty of Medicine.

RESULTS:

1. Histological and immunohistochemical results:

Hematoxylin and eosin stained sections of the brain of the control group revealed the hippocampus with its distinctive curved shape. The dentate gyrus appeared as V or U shaped structure that wrapped around the

end of the hippocampus proper. The *cornuammonis* (CA) was differentiated into fields. CA1 represented the first region of the hippocampalformation. The CA3 represented the last region of the hippocampus that dips into the dentate gyrus. The CA2 was the transitional zone connecting CA1 with **CA3** (Fig. 1).

1.1. The dentate gyrus:

Hematoxylin and eosin stained sections:

Examination of different stained sections of the brain of the control subgroup (I-b) revealed similar findings as compared to the control subgroup (I-a).

Hematoxylin and eosin stained sections of the dentate gyrus revealed that it was formed of three layers: polymorphic, granular, and molecular; among which, the granular layer was the predominant layer. It was formed mainly of aggregation of granule cells which appeared rounded to oval in shape with scanty rim of cytoplasm. Their nuclei were large, vesicular, with prominent nucleoli (Fig. 2A). Examination of stained sections of group II showed preservation of its three layers. However, few cells appeared dark, shrunken with pericellular spaces and the nuclei were deeply stained. Few dark cells were irregular and elongated(Fig. 2B). As regard group III, the three layers of the dentate gyrus were still preserved with apparent decrease in thickness of the granular layer. Also, few cells appeared dark, shrunken, with deeply stained nuclei and pericellular spaces(Fig. 2C).In group IV, examination of H&E stained sections showed that the majority of the granule cells were dark, shrunken, with pyknotic nuclei. Few normal granule cells were also seen (Fig. 2D).

Bielschowsky silver stained sections:

In Bielschowsky silver stained sections of the control group (group I), the granule cells showed its normal organization with regular outlines and dendrites (Fig. 3A). In both groups II and III, flame like neurofibrillary tangles were seen (Fig. 3B). As regard group IV, apparent increase in the number of neurofibrillary tangles was observed (Fig. 3D).

Immunohistochemical staining for GFAP:

Immunohistochemical staining for the control group (group I) showed the distribution of GFAP in astrocytes as dark brown dots or star shaped intracellular deposits (Fig. 4A). There was gradual apparent increase in the number of astrocytes and in the intensity of the reaction in groups II, III, and IV respectively (Figs. 4B, 4C, and 4D).

1.2. The *cornuammonis*:

Hematoxylin and eosin stained sections:

Examination of H&E stained sections of the different regions of the CA of the control group (group I) revealed that each region was formed of well-defined three layers: polymorphic, pyramidal, and molecular. Among these layers, the pyramidal layer was the main cell layer in the sections. This layer was formed of closely packed pyramidal cells which were arranged in a thickness of 3 to 4 rows of cells. The pyramidal cell appeared to have a triangular cell body with a large vesicular nucleus and a prominent nucleolus (Figs. 5A & 6A). In CA3, the pyramidal cells appeared larger in size than in CA1 with rounded to oval shapes (Fig. 7A).

As regard group II, H & E stained sections showed the preservation of the three layers of the CA in all regions. However, few cells appeared dark, shrunken, with pyknotic nuclei were seen (Figs. 5B & 6B). In few sections, pyramidal cells were disorganized and few dark cells appeared irregular and elongated with tapering end (Fig. 7B).

In group III, the three layers of the CA were also preserved in all regions with the presence of dark, shrunken cells with pyknotic nuclei (Figs. 5C & 6C). Some

sections showed apparent decrease in thickness of the pyramidal layer **(**Fig. 5C).Moreover, apparent increase in dark cells having irregular and elongated shapes was seen in some sections (Figs. 6C & 7C).While in group IV, there was loss of demarcation between the three layers of the CA in many sections. The pyramidal cells were widely separated and disorganized. Many cells appeared dark, shrunken, with pyknotic nuclei and pericellular spaces. Many irregular and elongated dark cells were seen (Figs. 5D, 6D & 7D). Apparent decrease in number of the pyramidal cells was also seen in some sections (Fig. 7D).

Bielschowsky silver stained sections:

In Bielschowsky silver stained sections of the control group (group I), the pyramidal cells showed regular outlines and dendrites (Fig. 8A). In group II, few flame like neurofibrillary tangles appeared in some sections (Fig. 8B).

As regard group III, apparent increase in flame like neurofibrillary tangles was noticed in all regions of CA (Fig. 8C). In group IV, further increase in the flame like neurofibrillary tangles was seen (Fig. 8D).

Immunohistochemical staining for GFAP:

Again, immunohistochemical staining of the control group (group I) showed the distribution of GFAP in astrocytes as dark brown dots or star shaped intracellular deposits (Fig. 9A). There was also gradual apparent increase in the number of astrocytes and in the intensity of the reaction in groups II, III, and IV respectively (Figs. 9B, 9C, and 9D).

2. Morphometric results and statistics:

A morphometric study was conducted and statistically analyzed. No significant differences were noted in the control group (I-b) in comparison with the control group (I-a).

2.1. Thickness of the granular layer of the dentate gyrus: (Table 1) and (Histogram 1)

The mean thickness of the granular layer of the dentate gyrus in group II revealed a non-significant decrease in comparison with the control groups. Moreover, the mean thickness of the granular layer of both group III and group IV recorded a highly significant decrease in comparison with the control groups and group II.

Furthermore, there was a non-significant decrease in group IV as compared with group III.

2.2. Thickness of the pyramidal layer of the *cornuammonis* (CA): (Table 2) and **(Histogram 2)**

CA1:

The mean thickness of the pyramidal layer of the CA1 in group II revealed a non-significant decrease as compared with the control groups. However, the mean thickness of the pyramidal layer of both groups III and IV recorded a highly significant decrease in comparison with the control groups and group II.

Furthermore, there was a non-significant decrease in group IV in comparison with group III.

CA2:

The mean thickness of the pyramidal layer of the CA2 in all experimental groups revealed a highly significant decrease in comparison with the control groups. As compared with group II, the mean thickness of the pyramidal layer of both groups III and IV recorded a significant decrease and a highly significant decrease respectively.

Moreover, there was a highly significant decrease in group IV as compared with group III.

CA3:

Also, the mean thickness of the pyramidal layer of the CA3 in all experimental groups revealed a highly significant decrease in comparison with the control groups. However, the mean thickness of the pyramidal layer of both groups III and IV recorded a non-significant decrease as compared with group II.

Furthermore, there was a non-significant decrease in group IV as compared with group III.

2.3. Mean area percentage of GFAP staining per microscopic field: (Table 3) and **(Histogram 3)**

Dentate gyrus:

The mean area percentage of GFAP staining per microscopic field of dentate gyrus of group II showed non-significant increase in comparison with control groups. However, the mean area percentage in both Tables and Histograms.

groups III and IV showed a highly significant increase as compared with control groups and group II.

Furthermore, there was a non-significant increase in group IV as compared with group III.

CA1:

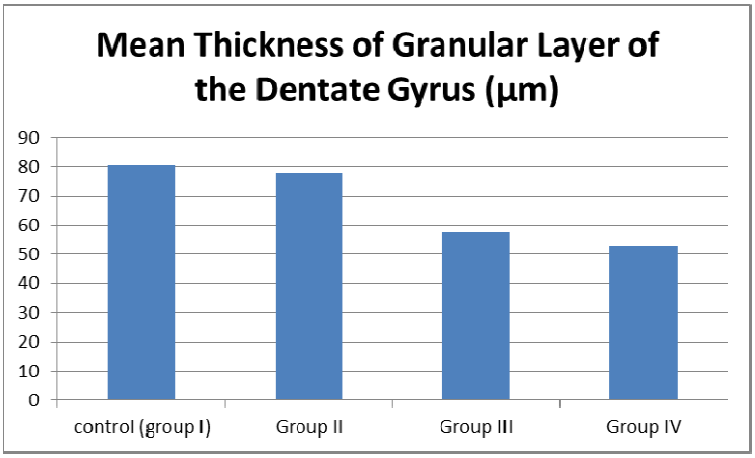
The mean area percentage of GFAP staining per microscopic field of CA1 region of group II showed also non-significant increase as compared with control groups. However, the mean area percentage in group III revealed a significant increase as compared with control groups and group II. Moreover, the mean area percentage in group IV showed a highly significant increase in comparison with control groups and group II.

Furthermore, there was a non-significant increase in group IV as compared with group III.

Table 1: Comparison of the mean thickness of the granular layer of the dentate gyrus (µm) ± SD between the experimental groups:

	Control (group I)	Group II	Group III	Group IV
Mean thickness of granular layer of the dentate gyrus ± SD	80.721 ± 3.75	77.96 ± 7.23	57.53 ± 4.72 (P<0.001)[a] (P<0.001)[b]	53.17 ± 5.5 (P<0.001)[a] (P<0.001)[b]

a) Highly significant decrease in comparison with control group.
b) Highly significant decrease in comparison with group II.



Histogram 1: Mean thickness of granular layer of the dentate gyrus (µm).

Table 2: Comparison of the mean thickness of the pyramidal layer of the *cornuammonis* (µm) ± SD between the experimental groups:

Mean thickness of pyramidal layer of the CA ± SD	Control (group I)	Group II	Group III	Group IV
CA1	73.74 ± 3.86	70.49 ± 7.31	45.88 ± 7.87 (P<0.001)[a] (P<0.001)[b]	42.55 ± 4.19 (P<0.001)[a] (P<0.001)[b]
CA2	75.99 ± 3.95	64.37 ± 4.62 (P<0.001)[a]	53.66 ± 7.9 (P<0.001)[a] (P=0.002)[c]	41.1 ± 4.72 (P<0.001)[a] (P<0.001)[b] (P<0.001)[d]
CA3	71.89 ± 8.88	46.89 ± 2.69 (P<0.001)[a]	45.96 ± 4.41 (P<0.001)[a]	44.4 ± 6.47 (P<0.001)[a]

a) Highly significant decrease in comparison with control group.
b) Highly significant decrease in comparison with group II.
c) Significant decrease in comparison with group II.
d) Highly significant in comparison compared with group III.

Histogram 2: Mean thickness of pyramidal layer of the *cornuammonis* (µm).

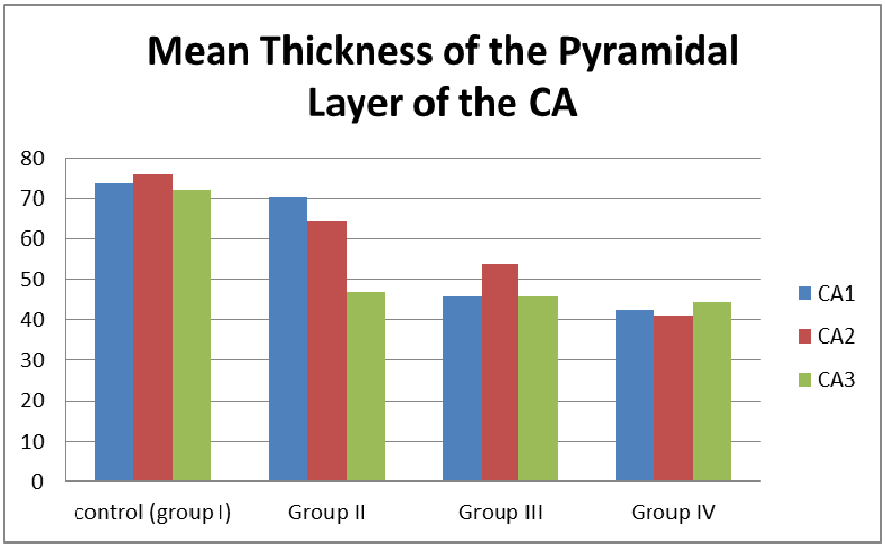
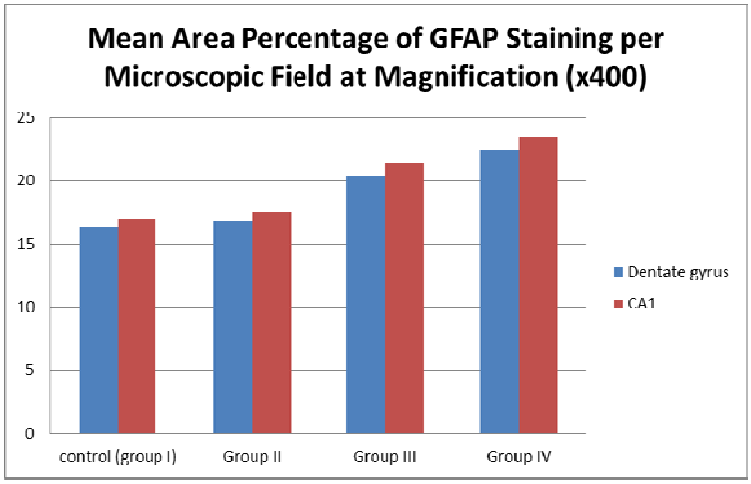


Table 3: Comparison of the mean area percentage of GFAP staining per microscopic field at magnification (x400) ± SD between the experimental groups:

Mean area percentage of GFAP staining per field ± SD	Control (group I)	Group II	Group III	Group IV
Dentate gyrus	16.36 ± 0.81	16.84 ± 0.99	20.48 ± 1.57 (P<0.001)[a] (P<0.001)[b]	22.48 ± 1.57 (P<0.001)[a] (P<0.001)[b]
CA1	17.03 ± 1.74	17.51 ± 1.31	21.49 ± 1.79 (P=0.00139)[c] (P=0.00136)[d]	23.47 ± 1.33 (P<0.001)[a] (P<0.001)[b]

a) Highly significant increase in comparison with control group.
b) Highly significant increase in comparison with group II.
c) Significant increase in comparison with control group.
d) Significant increase in comparison with group II.

Histogram 3: Mean area percentage of GFAP staining per microscopic field.



Figures and legends:

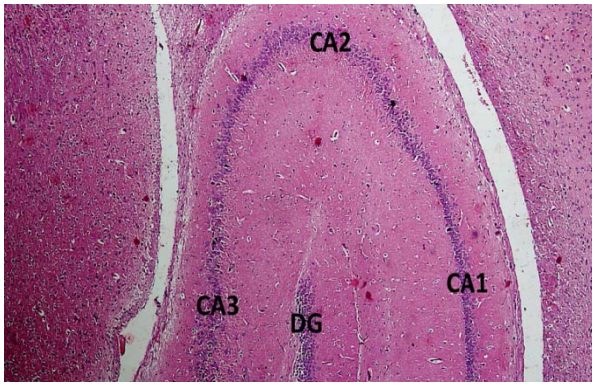


Fig. 1: A photomicrograph of a section in the brain of a rat from control group (group I) showing the parts of the hippocampal formation: regions of the cornuammonis (CA1), (CA2), and (CA3). Notice the dentate gyrus (DG). H&E, x40

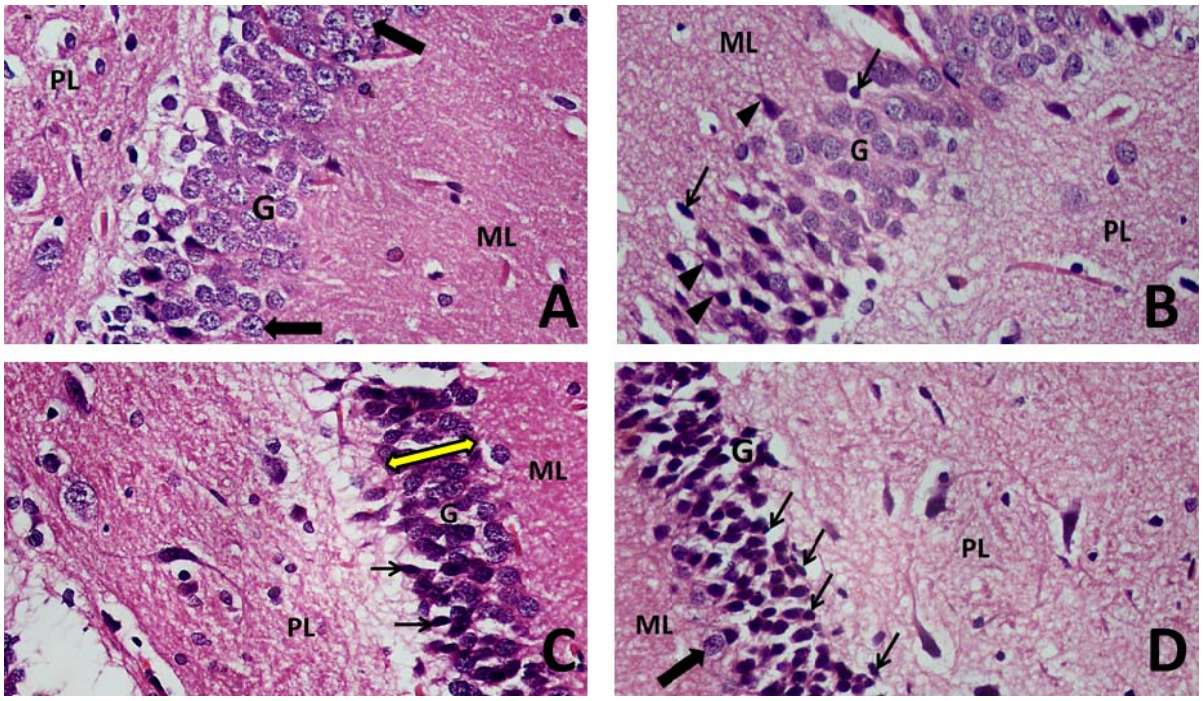


Fig. 2: Photomicrographs of sections in the hippocampus of rats showing the dentate gyrus formed of three layers: polymorphic (PL), granular (G), and molecular (ML).

(A) Group I (control): the granular layer is formed of aggregated granule cells. Each granule cell (thick arrows) appears rounded to oval in shape with large vesicular nucleus, prominent nucleolus, and scanty rim of cytoplasm.

(B) Group II: some granule cells (thin arrows) appear dark, shrunken, with pyknotic nuclei and pericellular spaces. Few dark cells appear irregular and elongated (arrow heads).

(C) Group III: dark shrunken granule cells with pyknotic nuclei are seen (thin arrows). Notice the apparent decrease in the thickness of the granular layer (double headed arrow).

(D) Group IV: most of the granule cells appear dark, shrunken, with pyknotic nuclei (thin arrows). Normal granule cells are hardly seen (thick arrow). H&E, x400

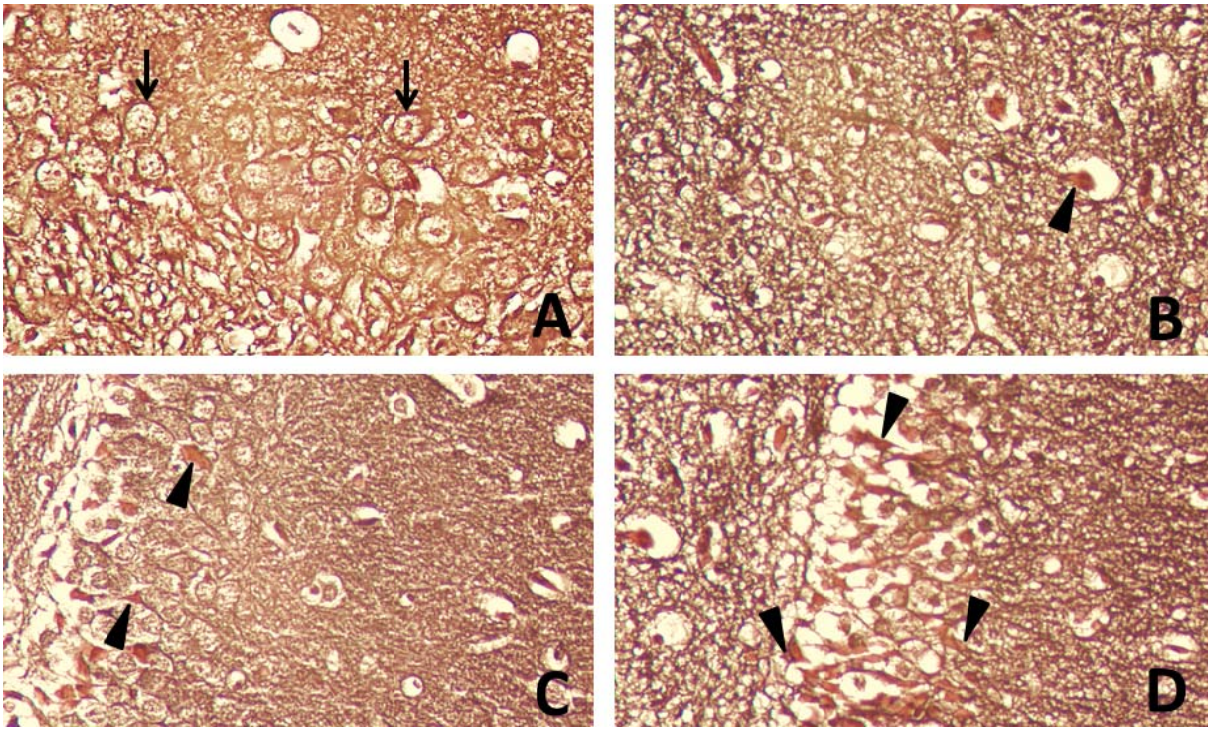


Fig. 3: Photomicrographs of sections in the hippocampus of rats showing the dentate gyrus. (A) Group I (control): the granule cells (arrows) show normal organization with regular outlines and dendrites. Notice the presence of flame like tangles (arrow heads) in (B) Group II, (C) Group III, and (D) Group IV. Bielschowsky silver, x400

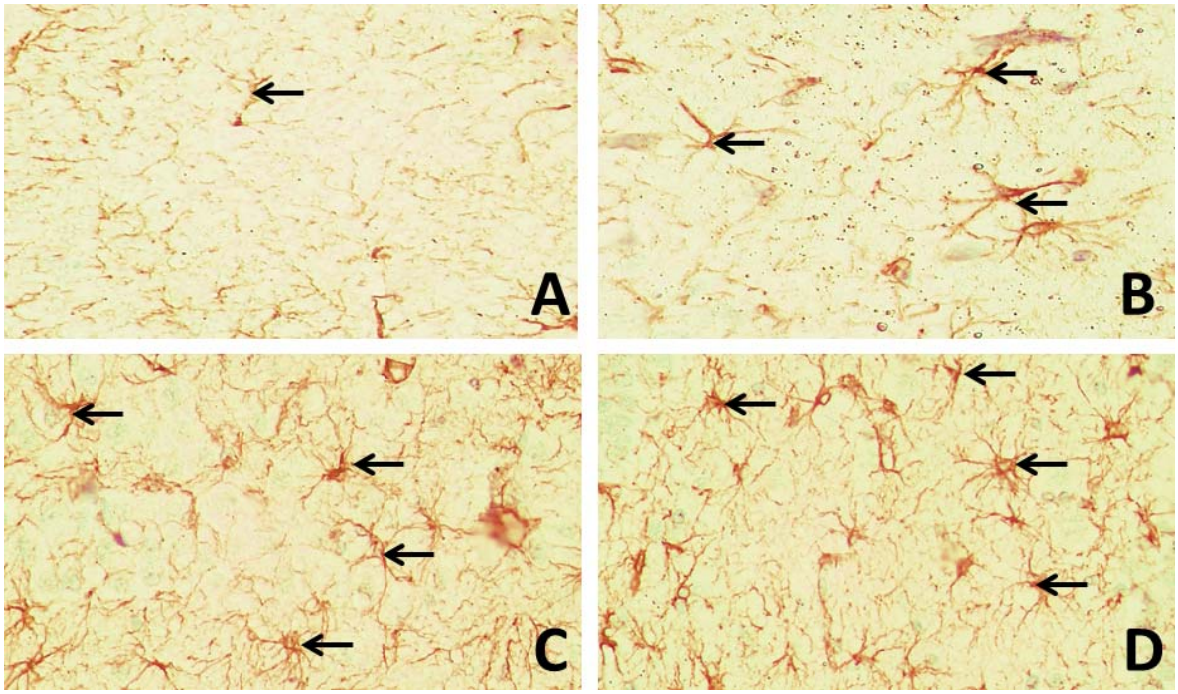


Fig. 4: Photomicrographs of sections in the hippocampus of rats at the region of dentate gyrus showing the brownish immunostaining of the astrocytes with ramifying processes (arrows). (A) Group I (control), (B) Group II, (C) Group III, and (D) Group IV. Notice the apparent increase in the number of astrocytes and in the intensity of the reaction. GFAP immunostaining, x400

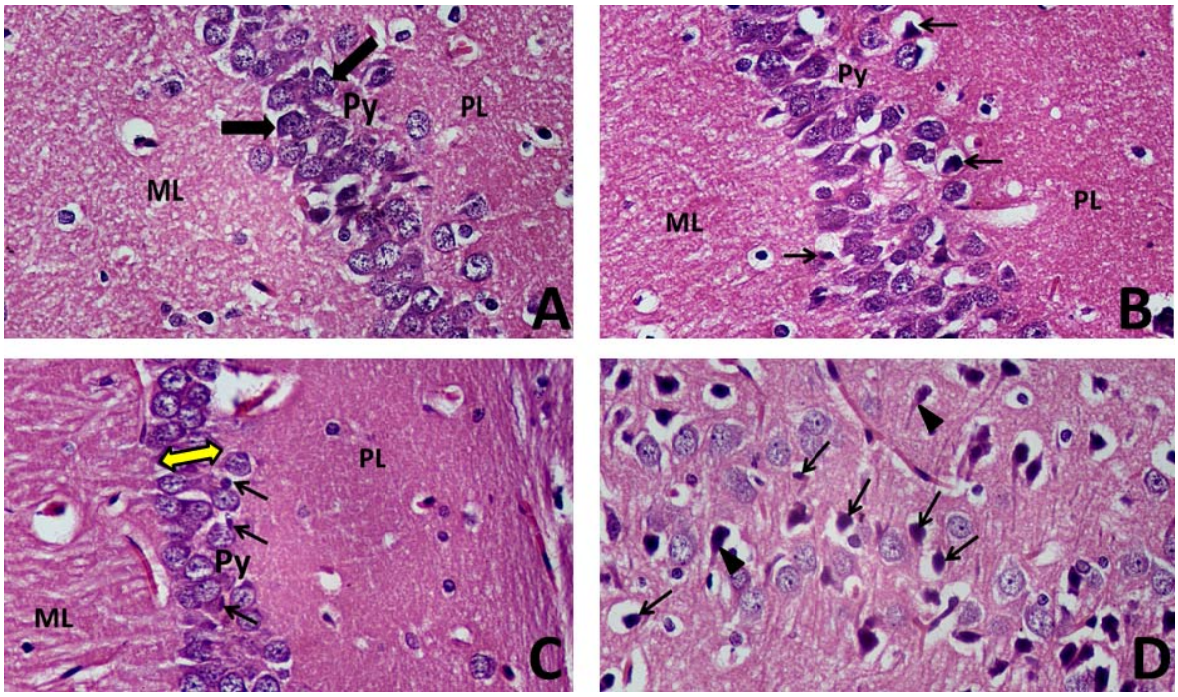


Fig. 5: Photomicrographs of sections in the hippocampus of rats showing the CA1 formed of three layers: molecular (ML), pyramidal (Py), and polymorphic (PL).

(A) Group I (control): The pyramidal layer is formed of closely packed cells which are regularly arranged in rows of 3 to 4 cell thickness. Pyramidal cells (thick arrows) appear large with scanty cytoplasm. Their nuclei appear vesicular with prominent nucleoli.

(B) Group II: few cells appear dark, shrunken, with pericellular spaces (thin arrows). The nuclei are pyknotic.

(C) Group III: some cells appear dark and shrunken (thin arrows). The nuclei are pyknotic. Note the apparent decrease in thickness of this layer (double headed arrow).

(D) Group IV: the pyramidal cells are widely separated and disorganized. Some cells appear dark, shrunken, with pericellular spaces (thin arrows). Note their pyknotic nuclei. Few dark cells appear irregular and elongated (arrow heads). H&E, x400

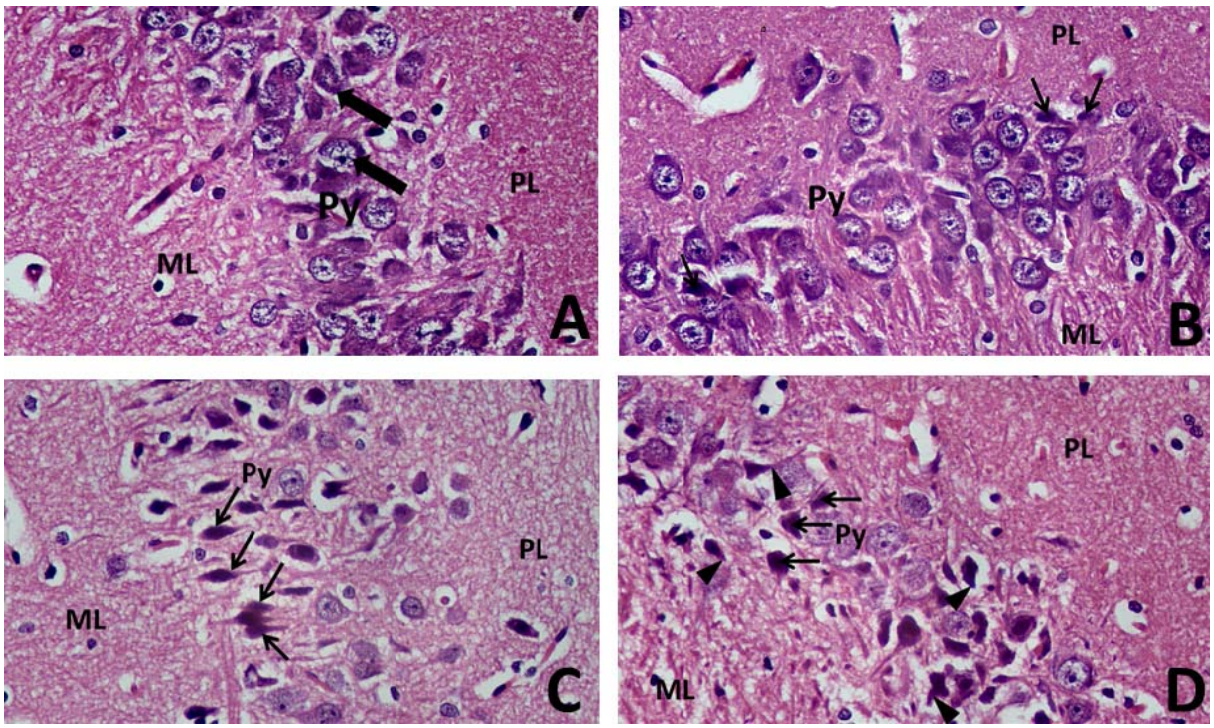


Fig. 6: Photomicrographs of sections in the hippocampus of rats showing the CA2 formed of three layers: molecular (ML), pyramidal (Py), and polymorphic (PL).

(A) Group I (control): the pyramidal layer is formed of closely packed cells which are regularly arranged in rows of 3 to 4 cell thickness. Pyramidal cells (thick arrows) appear large with scanty cytoplasm. The nuclei appear vesicular with prominent nucleoli.

(B) Group II: some cells appear dark, shrunken, with pyknotic nuclei (thin arrows).

(C) Group III: many cells appear dark and shrunken. Their nuclei are pyknotic. They appear irregular and elongated (thin arrows). Notice the disorganized pyramidal cells.

(D) Group IV: the pyramidal cells are disorganized. Many cells appear dark, shrunken, with pyknotic nuclei and pericellular spaces (thin arrows). Many dark cells appear irregular and elongated (arrow heads).

H&E, x400

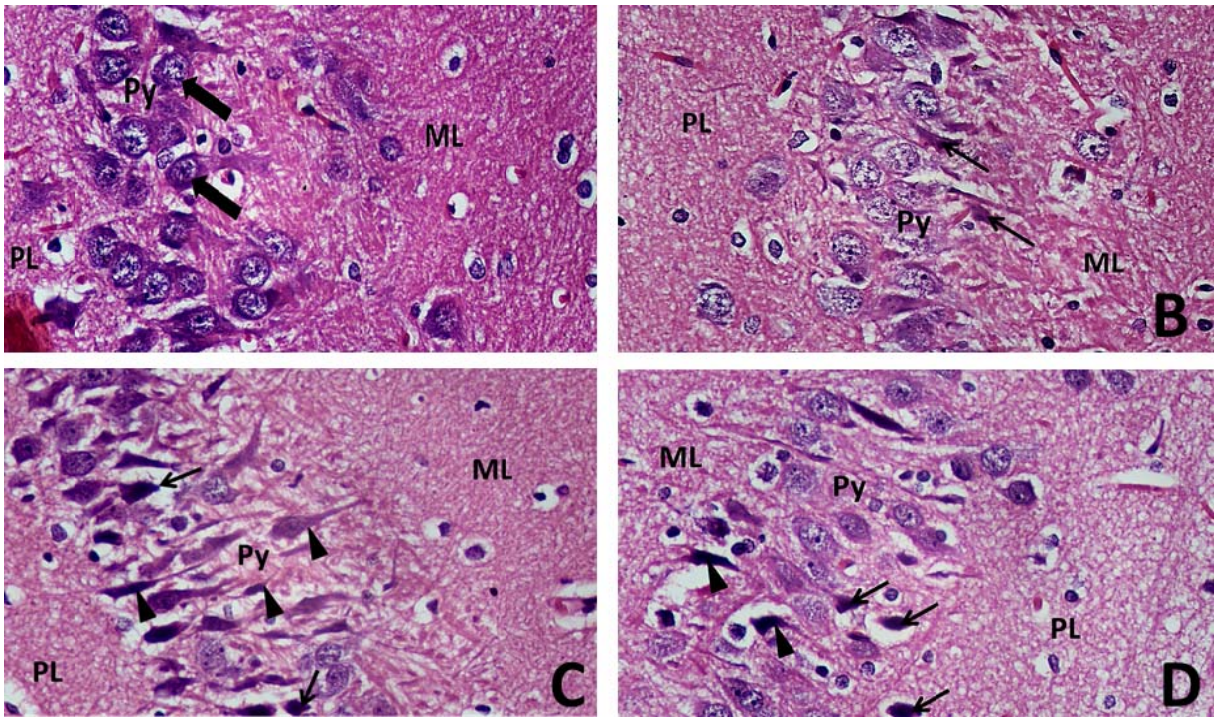


Fig. 7: Photomicrographs of sections in the hippocampus of rats showing the CA3 formed of three layers: molecular (ML), pyramidal (Py), and polymorphic (PL).

(A) Group I (control): the pyramidal layer is formed of large pyramidal cells (thick arrows) with rounded to oval shapes with scanty cytoplasm. Their nuclei appear vesicular with prominent nucleoli.

(B) Group II: few cells appear dark, shrunken, with pyknotic nuclei (thin arrows). Note the tapering end of the dark cells.

(C) Group III: the pyramidal layer shows many dark, shrunken cells with pyknotic nuclei and pericellular spaces (thin arrows). Some dark cells appear elongated (arrow heads).

(D) Group IV: the pyramidal layer is formed of widely separated cells. Some cells appear dark, shrunken, with pyknotic nuclei and pericellular spaces (thin arrows). Some dark cells appear elongated with tapering end (arrow heads).

H&E, x400

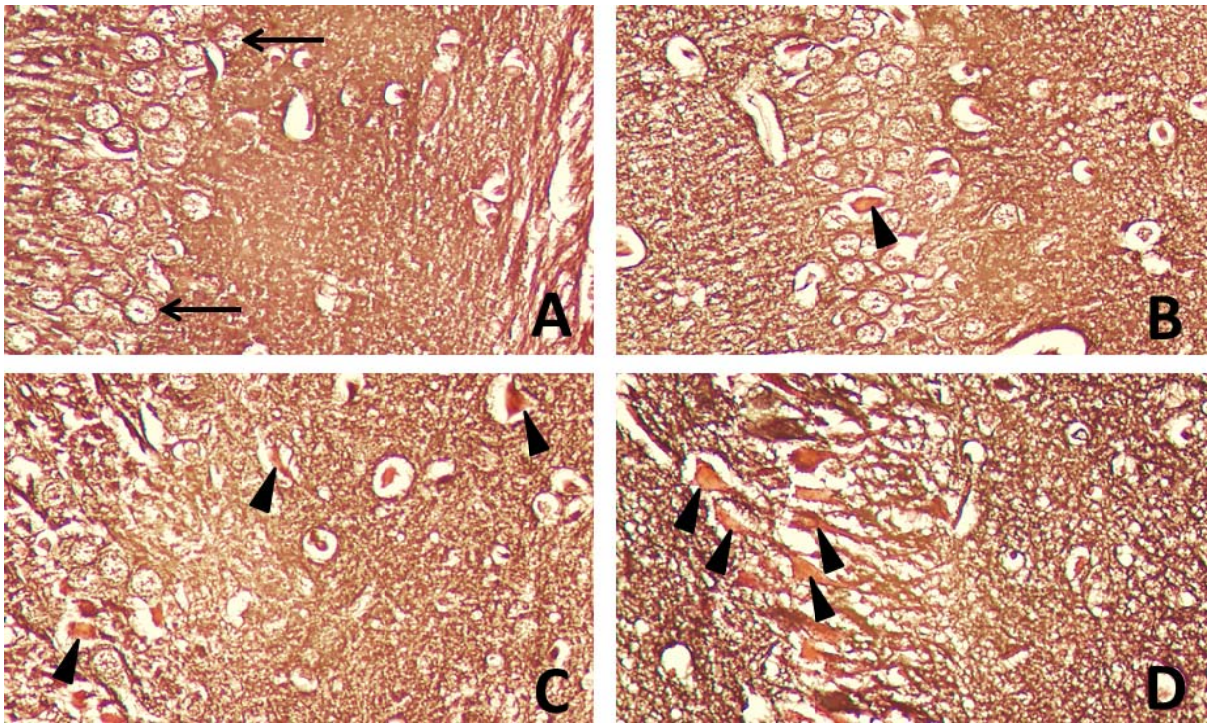


Fig. 8: Photomicrographs of sections in the hippocampus of rats showing the region of CA1. (A) Group I (control): the pyramidal cells (arrows) show normal organization with regular outlines and dendrites. Notice the presence of flame like tangles (arrow heads) in (B) Group II, (C) Group III, and (D) Group IV. Bielschowsky silver, x400

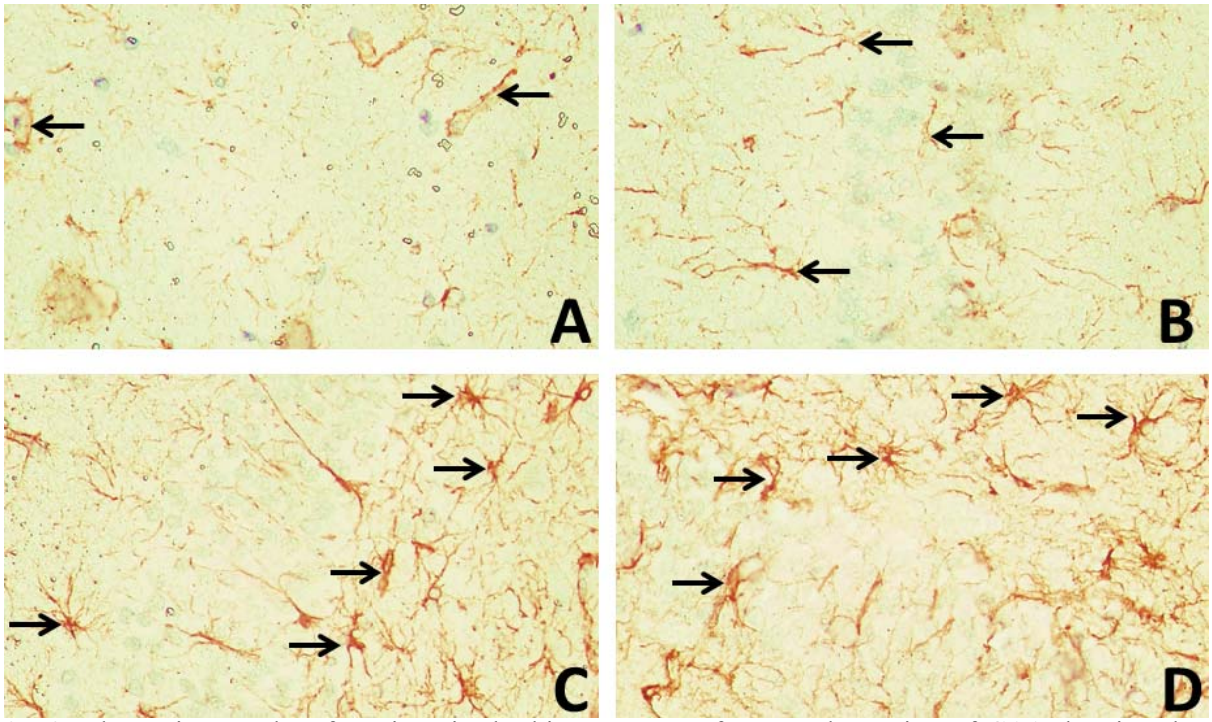


Fig. 9: Photomicrographs of sections in the hippocampus of rats at the region of CA1 showing the brownish immunostaining of the astrocytes with ramifying processes (arrows). (A) Group I (control), (B) Group II, (C) Group III, and (D) Group IV. Notice the increase in the number of astrocytes and in the intensity of the reaction. GFAP immunostaining, x400

DISCUSSION:

The present work provided an evidence of the detrimental effect of chloroquine administration on the hippocampal neurons in adult male albino rats. This evidence was obtained on both histological and immune-histochemical levels. The results obtained from this study showed the signs of neuronal damage in the hippocampi of chloroquine treated rats compared to those of control rats. Examination of different components of the hippocampal formation showed dark, shrunken cells, with pyknotic nuclei and large pericellular spaces. These degenerative signs appeared in group II, and increased in both groups III and IV providing evidence that these changes were duration dependent.

In the present study, male albino rats were used to avoid the female hormonal effect as estrogen hormone was suggested to enhance cell proliferation which may mask the studied adverse effects[35].

The stains employed in this study are classically used to examine signs of neuronal injury in conditions of memory affection as in cases of AD[36]. Hematoxylin and eosin staining was utilized to examine the morphology of pyramidal and granular nerve cells[37]. Subsequently, Bielschowsky silver stain was used to detect neurofibrillary tangles (NFTs)[38]. Finally, Immuno-histochemical localization of GFAP was employed to study the response of astrocytes to neuronal injury as well as their distribution[34].

The current study included all regions of the hippocampus as regard H&E staining and most of the morphometric measure-ments. As regard Bielschowsky silver and immunohistochemical stains, the study focused on the area's most involved in memory; the dentate gyrus and *cornuammonis* (CA1) of the hippocampus proper. The dentate gyrus plays an important role in memory especially long term potentiation (LTP) and long-lasting strengthening of synaptic connections following repeated stimulation. This role is attributed to the function of mossy fibers in the memory pathway[39]. Furthermore, the neurons of CA1 take a major part in processing of the hippocampus-dependent memory[40] and LTP[41].

In the present study, neural degeneration in the form of cellular shrinkage and nuclear pyknosis was detected in H&E stained sections. This was in accordance with neuro-pathologically studied cases of AD [42] that revealed neuronal death in the hippocampus, which was recorded to be the most severely involved region in the brain in AD [43].

The results of the present work revealed the presence of irregular and elongated dark cells resembling those having NFTs of tau protein. These were observed in H&E stained sections and confirmed by Bielschowsky silver staining. NFTs were seen in the neurons of all chloroquine treated rats that increased with duration of the treatment. In support of results of the present study, researchers stated that in neuro-degenerative diseases that are called tauopathies including AD, tau protein is hyperphosphorylated with subsequent aggregation into bundles of filaments [44]. Neurofibrillary degeneration of hyper-phosphorylated tau protein is apparently required for the clinical expression of AD and related tauopathies[45]. Moreover, non-invasive staging of AD needs multi-parametric quantitative MRI imaging technique to classify regions of high and low NFTs density [46].

In the present work, a non-significant increase in the density of astrocytes in the two weeks chloroquine treated group was observed. Also, a significant increase was seen in both groups III and IV. So, this increase was found to prevail by increasing duration of chloroquine treatment. In consistence with these findings, it was reported that late stages of neuro-degenerative diseases such as AD show astrogliosis that was routinely detected in

post-mortem human tissues and is also observed in animal models of these diseases. This astrogliosis is characterized by hypertrophy of astroglial cells and accompanied by upregulation of the expression of GFAP[47].

All these results were supported on morphometric level. There was a highly significant decrease in the thickness of granular layer of dentate gyrus and pyramidal layer of CA especially in both groups III and IV as compared with control groups.

So, the results of the present study collectively were similar to those typically described in the pathological features of neurodegenerative diseases especially AD[48]. These results were particularly more prominent in groups III and IV.

The results of the present study go in agreement with results of other studies that revealed the neurotoxic effects of chloroquine.

Chloroquine has been reported to cause damage to both spinal cord and brain of fetus, including damage to sense of balance and hearing in animal studies [49].

Adjene and Adenowo[28] have recorded the adverse effects of chloroquine on the inferior colliculus in rats and stated that these adverse effects may underlie the possible neurologic symptoms, such as tinnitus, that previously reported by Manolette[50] following chloroquine treatment.

Mahon et al. [51] and Yamada et al**.** [52] have reported that chloroquine neutralized the cellular acidic organelles such as lysosomes resulting in the elevation of PH with resultant inhibition of the acid dependent hydrolases of these organelles. This mechanism was believed to cause the chloroquine induced retinopathies.

Also, it was recorded that antimalarial drugs disrupt both serotonin biosynthesis and function, giving important insight to the action of these drugs on mammalian cells and development of psychiatric side effects[53].

More recent study concluded that chloroquine produced oxidative stress in the brain, liver, and kidney of rats and adversely affected the DNA[31].

On the other hand, results of the present study were contrary to those of other researchers who recorded the neuro-protective effect of chloroquine in certain situations.

During brain tumor therapy, Naoshi et al.[54] stated that chloroquine was useful to reduce the toxicity of tumor therapy for normal brain without inhibiting antitumor efficacy and increased the therapeutic window for brain tumor therapy.

Chloroquine was believed to inhibit the functions and proliferation of glial cells in the hippocampus and cerebral cortex of rats alleviating the seizure activities in cases of drug induced seizures [24]. Other studies revealed that chloroquine had a potential therapeutic role in both chronic and acute neurological disorders, including brain ischemia and AD[25&26]. Moreover, chloroquine attenuated autophagy and inflammation in rat hippocampus following traumatic brain injury[27].

Limitations of the study:

Although the results of this study were informative about the deleterious effect of chloroquine on the structure of the hippocampus in rats, it has certain limitations. This study is one of the rare studies that conducted to assess the effect of chloroquine on healthy hippocampus on histological, immunohistochemical, and morphometric levels. The study also included different durations of treatment but with the same daily dose lacking the comparison between different doses of chloroquine. So, it is recommended to conduct further studies including different

doses of the drug with examination of different parts of the nervous system.

Conclusion:

It was concluded that oral administration of chloroquine caused duration dependent neuronal damage in the hippocampus of male adult albino rats giving a possible explanation for chloroquine induced neuropsychiatric adverse effects. This could demonstrate valuable for individuals who deal with chloroquine prescriptions.

Conflict of interest:

We declare that we have no conflict of interest, intent of financial gain, or commercial associations regarding this research.

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هل تناول الكلوروكين عن طريق الفم يؤثر على الحصين في الفئران؟ مفتاح للآثار السلبية العصبية والنفسية الناجمة عن تناول الكلوروكين

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خلفية البحث: يستخدم الكلوروكين على نطاق واسع في الطب. ويعد الاستخدام الرئيسي له هو علاج الملاريا.وتعد التقارير المتعلقة بالآثار الجانبية النفسية للكلوروكين نادرة. مع ذلك، فإن قائمة الاضطرابات العصبية والنفسية التي يسببها الكلوروكين تشمل مجموعة واسعة من الأعراض.

الهدف من البحث: في هذه الدراسة، تم تقييم تأثيرتناول الكلوروكين عن طريق الفمعلى الحصينفي الفئران.

المواد والطرق المستخدمة: تم تقسيم سبعة وعشرينمن ذكور الفئران البيضاء البالغة عشوائيًا إلى أربع مجموعات. المجموعة الأولى (المجموعة الضابطة): تم تقسيم تسعة فئران إلى مجموعتين فرعيتين فرعية١- أ و١ – ب.المجموعة الثانية (الكلوروكين لمدة أسبوعين): تلقت ستة فئران ٤مل من محلول الماء المقطر يوميًا يحتوي على الكلوروكين بجرعة ٨٠ مغ / كغمنوزن الجسم عن طريق الفم لمدة أسبوعين. المجموعة الثالثة (الكلوروكين لمدة ثلاثة أسابيع): تلقت ستة فئران الكلوروكين كما في المجموعة الثانية لمدة ثلاثة أسابيع. المجموعة الرابعة (الكلوروكين لمدة أربعة أسابيع): تلقت ستة فئران الكلوروكين كما في المجموعة الثانية لمدة أربعة أسابيع. بعد التضحية بالفئران ، تم استخراج الحصين ومعالجته للفحص بالمجهر الضوئي. تم صبغ قطاعات البارافين بصبغتي هيماتوكسلين وايوسين وبيلشوفسكي الفضة وتم إجراء صبغ مناعي لفحص توزيع الخلايا النجمية.

النتائج: كشف فحص مناطق مختلفة من تكوين الحصين عن وجود خلايا داكنة منكمشة ، مع أنوية داكنة ومساحات كبيرة حول الخلايا في جميع المجموعات المعالجة. و شوهدت التشابكات العصبية الليفية في مختلف المقاطع المصبوغة. علاوة على ذلك ، لوحظت زيادة في كثافة الخلايا النجمية. ومن الناحية القياسية، كان هناك انخفاض في سمك كل من الطبقة الهرمية لقرن آمون والطبقة الحبيبية من التلفيف المسنن في جميع المجموعات المعالجة بالمقارنة مع المجموعة الضابطة. ظهرت كل هذه التغييرات في المجموعة الثانية ، وكانت أكثر وضوحًا في المجموعتين الثالثة والرابعة.

الاستنتاج:نتج من هذا البحثأن تناول الكلوروكين عن طريق الفم تسبب في أضرار عصبية اعتمدت على مدة العلاج في حصين الفئران مما يعطي تفسيرًا محتملًا للآثار السلبية العصبية والنفسية الناجمة عن تناول الكلوروكين.