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Effect of Oral Administration of Paraquat Pesticide on the Hippocampus and Substantia Nigra in Wistar Rats' Brains

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Abstract: This study aimed to detect the neurohistological damages of chronic exposure to low levels of pesticide (paraquat) in the hippocampus, and substantia nigra in Wistar rats' brains. The neurotoxic effects of acute poisoning are well established but the possibility that low level exposure causes different diseases is controversial. It is important to get a clear answer to this question as more individuals are at risk of low level exposure than acute poisoning. The anatomical and histological of current study to affected brains showed cells display the cytological changes of herbisecticides-lesioned brain tissue, such as a significant decrease in the size of the brain was observed, as most of its external features disappeared. in addition, we detected vacuolization around cells that degenerated because many reasons like apoptosis or necrosis, and the intracellular neurofibrillary tangles were observed at many regions such as the hippocampus and substantia nigra. Moreover extracellular amyloid plaques take fibers form were detected. we also observed degenerated in CA1, CA2 and CA3 regions (molecular layer, polymorphic layer and pyramidal layer) by pigmenting degenerated neurons with silver nitrate with increased astrocytes of glia cells.

Keywords: Paraquat, Hippocampus, Substantia Nigra, Neurotoxic, Cell Degeneration, Male Wistar Rat.

تأثير التجريع الفموي للمبيد العشبي (الباراكوات) على الحصين والمادة السوداء في أدمغة جرذان الويستر

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المستخلص: هدفت هذه الدراسة إلى تحديد التأثيرات النسيجية العصبية من خلال التعرض المزمن للمستويات المنخفضة من المبيد العشبي(الباراكوات) في الحصين والمادة السوداء في أدمغة جرذان الويستر، نُشرت بشكل جيد التأثيرات السمية العصبية للمستويات الحادة والذي يدل على إمكانية التأثير المنخفض على أمراض مختلفة. أظهرت الدراسة الحالية تشريحياً ونسيجياً للأدمغة المتأثرة التغيرات الخلوية المميزة للمبيدات العشبية، تضمنت تناقص معنوي في حجم الدماغ مع اختفاء معظم ملامحه الخارجية، بالإضافة إلى

تحديد الفضوات حول الخلايا التي تراجعت لعدة أسباب كالموت الخلوي المبرمج والتنخر، وظهور التشابكات الليفية العصبية داخل الخلايا في عدة مناطق مثل الحصين والمادة السوداء. بالإضافة إلى تحديد الصفيحات الأميلوئيدية خارج الخلايا على شكل ألياف، كما لوحظ التراجع العصبي في مناطق قرن آمون 1،2و3 (الطبقة الجزيئية، الطبقة متعددة الشكل، والطبقة الهرمية) بوساطة تصبغ العصبونات المتراجعة باستخدام نيترات الفضة مع ملاحظة ازدياد عدد الخلايا الدبقية النجمية.

الكلمات المفتاحية: باراكوات، الحصين، المادة السوداء، التسمم العصبي، التراجع الخلوي، ذكور جرذان الويستر.

1. Introduction:

Parkinson's(PD) and Alzheimer's(AD) diseases are the two most common neurodegenerative diseases. The effects of paraquat on human health have been growing as they are increasingly used throughout the world for avariety of agricultural, industrial and domestic purposes. Many species tested include the companion animals (dogs, cats etc.) and livestock (pigs, sheep, geese etc.) were also reviewed animals are used to model (PD) and (AD) to research the pathogenesis and added to discover new potential medicines contributed by Herbicides paraquat [21]. In all cases studied to date, lesions were found most consistently in the Parahippocampal(CAP1, CAP2) of the hippocampus, amygdala, substantia nigra, and neocortex. Other brain areas affected include basal ganglia [19]. Neurodegenerative diseases, particularly Parkinson's and Alzheimer's, have common features including: protein accumulation, cell death with mitochondrial involvement and oxidative stress and degeneration of neuromelanin, in the neurons of Substantia Nigra (SN), which is an organic polymer produced by dopamine metabolism, It is primarily showed contain large amounts of neuromelanin and the concentration of neuromelanin increases with age, while it is markedly decreased in PD patients. Moreover, PD-like pathologies characterized by significant neuronal loss and the presence of Lewy bodies composed of highly phosphorylated a-synuclein have been noted in three months treatment animals. Parkinsonism, is accompanied by degeneration of the nigra dopaminergic system, with neuronal loss and reactive gliosis substantia nigra found at autopsy [15]. In idiopathic parkinson's disease (PD), α synuclein accumulates neuronnal perikarya (lewy bodies) and neuronal processes (lewy neurites) [25]. PD is clinically depicted by severe motor symptoms, including rigidity, postural instability, a resting tremor, and bradykinesia. PD pathology is characterized by progressive degeneration and the loss of dopaminergic (DA) neurons in the substantia nigra (SN) pars compacta. Moreover, the deposition of α-synuclein as insoluble and toxic aggregates are characteristic hallmarks of PD. Regarding AD, patients suffer from irreversible memory loss, progressive cognitive impairment, language disorder, and impairment in their visuospatial skills due to the degeneration of the hippocampal and cortical neurons and extracellular amyloid plaques. The earliest understanding of the disease pathology of PD and AD focuses on neuronal degeneration and consecutively observed inflammation, which is likely to be activated by the damaged neurons [6, 23]. In previous studies, lesions were consistently found in the CAP1 and CAP2 of the hippocampus, amygdala, SN, and neocortex. Neurodegenerative diseases, including PD and AD, have common features, including the following: protein

accumulation, cell death with mitochondrial involvement, oxidative stress, and degeneration of neuromelanin, which is an organic polymer that is produced by the dopamine metabolism [12, 20, 24]. The neurons of the SN contain large amounts of neuromelanin, and the concentration increases with age. However, this was markedly decreased in PD patients. However, PD-like pathologies are characterized by a significant neuronal loss and the emergence of Lewy bodies that are composed of highly phosphorylated a-synuclein, which were observed in the treatment animals after three months [8, 15]. This study aims to found the degenerative effects of chronic exposure to Paraquat on the dopaminergic system in rats and detect the possible contributing mechanisms.

2. Materials and methods:

Thirty male Wistar rats were maintained on a 12-hour light/12-hour dark schedule and given unlimited access to rat food and tap water in the Animal Department, Faculty of Science at the University of Aleppo. The animals were divided into two groups. The first group was orally given an aqueous solution of Paraquat (0.1 mg\g body weight) for four months, and the second group was given water without paraquat. Their brains were then removed from their skulls and stored for at least three days in the fixative (formaldehyde 4%). Next, each brain was cut using a microtome into a series of sections that were 10 µm–20 µm thick. The serial sections (10 µm–20 µm thick) were then stained using different stains. The first stain was hematoxylin-eosin (H&E), which is distinguished by its good staining cell structures, such as cytoplasm and nuclei. The second was Cresyl violet, which is used for the central nervous system, including the nerve cell contents and Nissl substances. In addition, Colgi and Belcshowsky stains were used for the dendrites of neurons. The staining method was carried out according to the following stages: according to procedure of (Harris-1990) [31].

1- dissolving paraffin from textile preparations using an electric heater at a temperature of 50° C–52° C; 2) transferring the slides to two baths of xylol for five minutes for each bath; 3) transferring the slides to a series of graduated concentrations of alcohol (100%, 90%, and 70%); and 4) washing them in distilled water to return water to the samples for five minutes per bath. In the H&E staining method, the hematoxylin was used first for five minutes, and then the slides were washed in distilled water and placed in the eosin stain for one minute. Later, the samples were transferred from the colorant to the distilled water and then to a series of gradual concentrations of alcohol (70%, 90%, and 100%) to dehydrate. They were kept in each concentration for one minute, and then they were transferred to two baths of xylol for two minutes each. The tissue preparations were covered in glass screens after an adhesive substance (Canada balsam or DPX) was applied to preserve them. Then, they were placed in a special dryer at a temperature of 37° C until the adhesive dried out.

2.1. Colgi and Beilcshowsky Stain Methods:

If the samples were large, they were divided into small pieces of 4 ml to 5 ml. Once the samples had been washed after the fixer and their tissues had been dried using blotting paper, they were placed in the potassium dichromate 2% solution for two days in the dark. After drying, the samples were transferred to the second solution (silver nitrate 2%) for two days. In this staining method [17], the double impregnation or Ramon Cajal's staining technique was used. Afterwards, the samples were transferred to 1% oxalic acid and washed in 5% sodium thiosulfate. The samples passed an alcohol chain after being dried with blotting paper (70-90-100-Xylol). Later, the sample passed through the paraffin chain, and they were cut into 20 µm–50 µm-thick sections. The slides passed a chain of alcohol xylol (100-90-70-water-70-90-100-xylol). Finally, there was a microscopic examination of the slides [21].

2.2. Congo Red Stain Method:

In this stain, the sections were deparaffinized, and the water was removed. Then, they were stained in Congo red for 30–60 minutes, rinsed in distilled water, and passed through alkali alcohol for 5–10 seconds. The sections were then washed in distilled water for five minutes, counterstained in hematoxylin for 30 seconds, and washed in distilled water for one minute. Later, the sections were dipped in ammonia water for 30 seconds to turn them blue (the ammonia water was prepared by adding a few drops of ammonium hydroxide to tap water and mixing carefully). The sections were then rinsed in distilled water for five minutes and dehydrated using 95% alcohol, 100% alcohol, and Xylol[29].

3. Results:

The incidene of lesions produced by paraquat was summarized in histological and behavioural changes were seen in the region of the hippocampus and substantia nigra in midbrain and brain stem. Thus an decrease of brain size was found due to lysis most of cells in neocortex (fig 1).

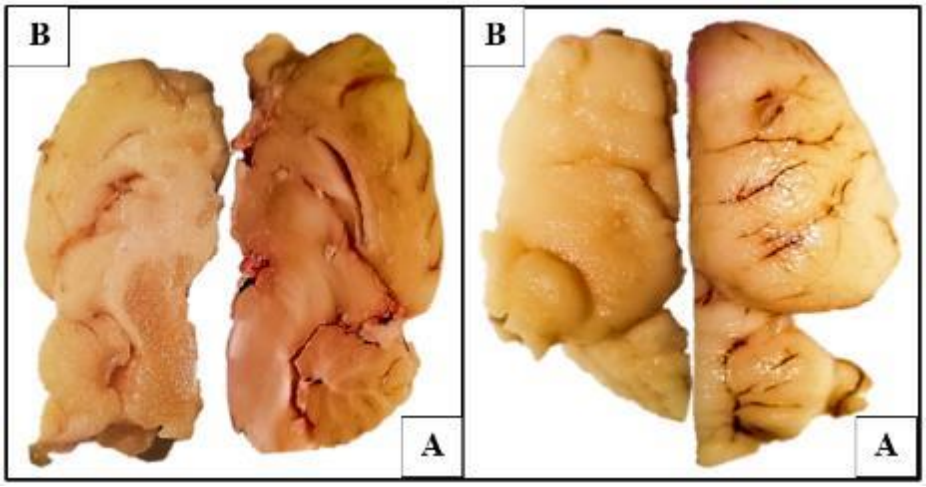

Fig. (1) sagittal section in a wistar rat brain of group (0.1 mg/ kg body weight paraquat once daily for four successive months). (B) showing an internal and an external side view that clearly shows the

decrease in the size in addition to blurring of most of the features of their brains, compared to the control (A).

Also, the neurofibrillay tangles were documented clearly in compacta pars region of the substantia nigra and extracellular neuritic AD plaques are readily observed between dopaminergic neurons, and presence of abnormally aggregated NFTs together with damaged neuronal cells (fig 2).

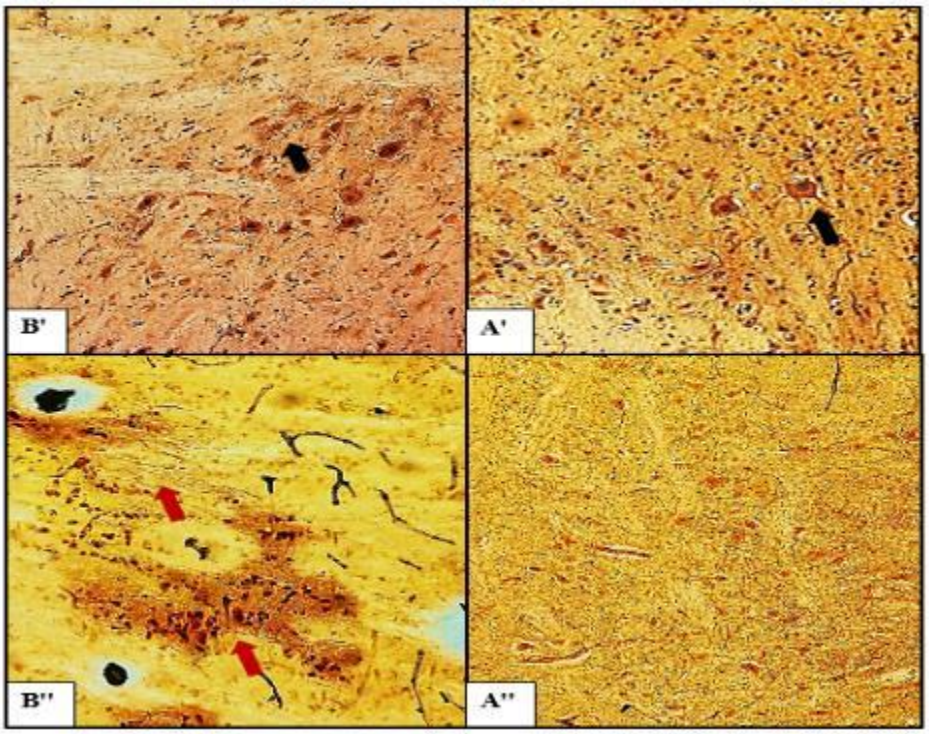

Fig. (2) A'& A": control, **B'& B":** group of (0.1 mg/ kg body weight paraquat once daily for four successive months). Bielschowsky silver nitrate stained sections of the rat substantia nigra using pretreatment with potassium permanganate and oxalic acid. **(B')** neurofibrillary tangles in compacta pars (black arrow) compring to control **(A')** x600, and **(B")** extracellular Neuritic AD plaques are readily observed between dopaminergic neurons (red arrow) compred to the control **(A")** x400

we also noticed some of necrotic neuronal cells were visibled in the region of compacta pars, and increased of neuromelanin in degenerated neurons (fig3)

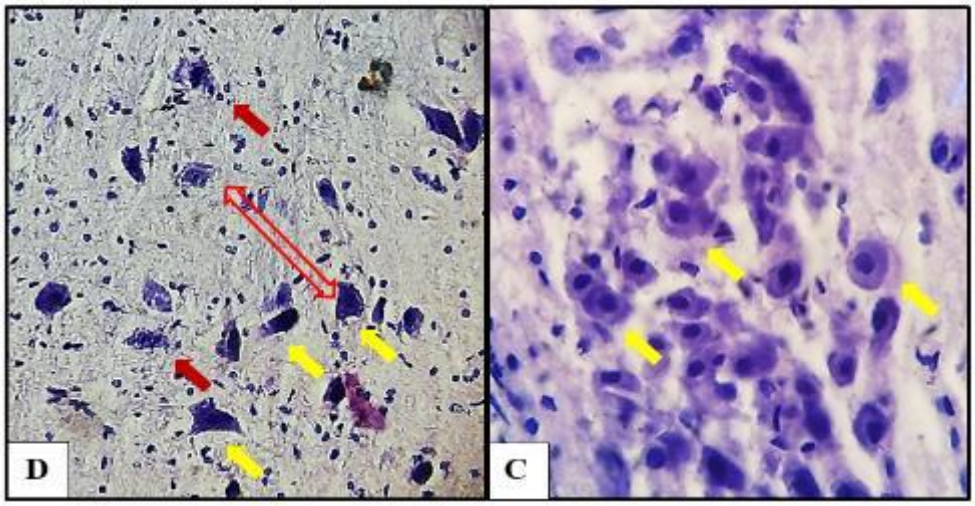

Fig. (3) cresyl violet stained sections of the rat substantia nigra of group (0.1 mg/ kg body weight paraquat once daily for three successive months) showing **(D)** more extra cellular space (lift-right arrow), A number of necrotic neuronal cells are visible in the region of compacta pars (arrow), and increase of neuromelanin in degenerated neurons (arrow). than in the un affected group **(C)** x600.

The most important symptoms of Alzheimer's disease were observed in this area (dentate gyrus), such as necrotic neuronal elements with bloated cytoplasm and pyknotic nuclei (fig4).

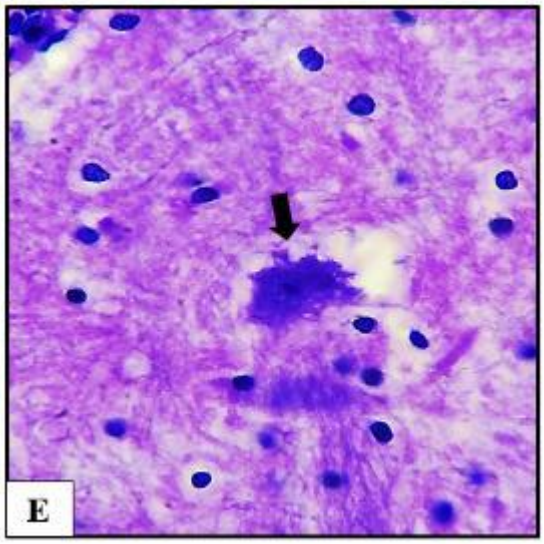

Fig. (4) Hematoxyline and Eosin stained sections of the four-six months wistar rats dentate gyrus in the hippocampus of group (0.1 mg/ kg body weight paraquat once daily for 4 successive weeks) showing **(E)** neuronal elements are necrotic with bloated cytoplasm and pyknotic nuclei (arrow).x1000

In addition to the increase of microglial cells was seen in the hippocampus and substantia nigra of 80% of rats given paraquat orally, and visible damage to neurones was clear. The affected cells also were vacuolated (fig 5).

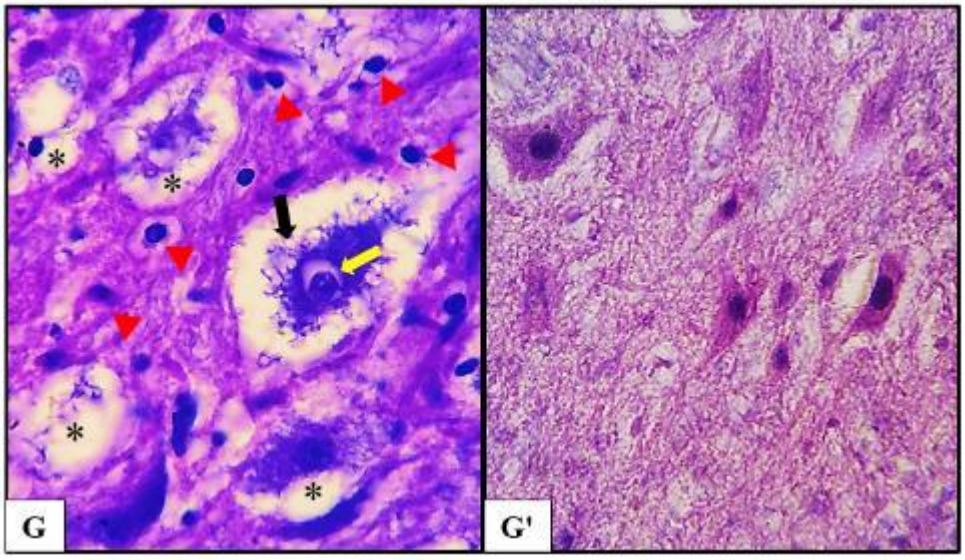

Fig. (5) Crysel violet stained sections of the rat Hippocampus (DG) of group (0.1 mg/ kg body weight paraquat once daily for three successive months) showing **(G)** Neurofibrillary tanglesand strange halo arround the neuclei in the centre (yallow arrow) and presence of abnormally aggregated NFTs together with damaged neuronal cells (black arrow). There is an increase in glial cells (head arrow). In these cases, the damaged cells also become vacuolated (*). Compared to the control **(G')**x 1000

Morever, the examination of brains that treated with paraquat the Amyloid plaque were found deposits in compacta pars region between neurons are heavily stained in red by Congo redz method, Congo Red counterstaining was observed between crossed polarization fibers confirmed the amyloid nature of the central core. (fig 6)

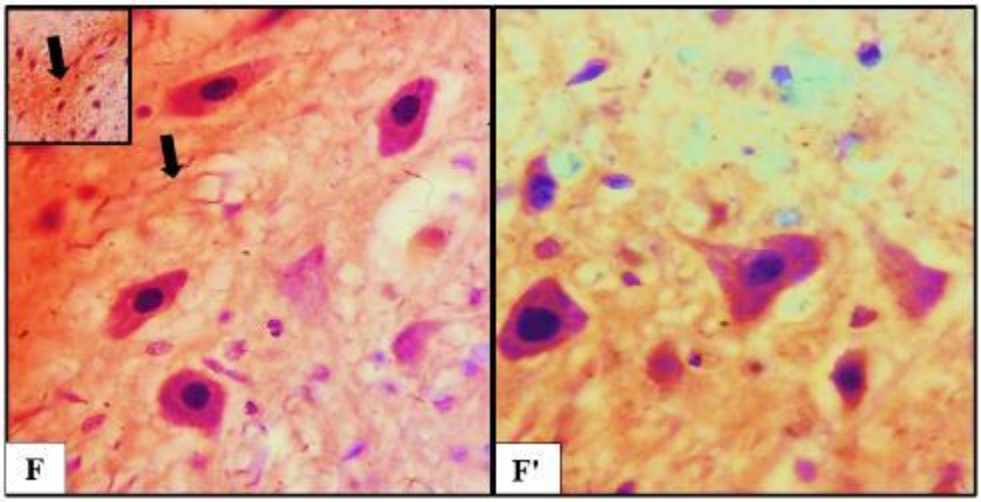

Fig. (6) Congo red stained sections of the rat substantia nigra of group (0.1 mg/ kg body weight paraquat once daily for three successive months) showing **(F)** Amyloid plaque deposits in compacta pars region between neurons are heavily stained (black arrow) compared to the control **(F')** x1000.

And the nuclei were displayed the uniform dispersion of hetero chromatin characteristic of neurons and have prominent nucleolus in the hippocampus (CA3) (fig7).

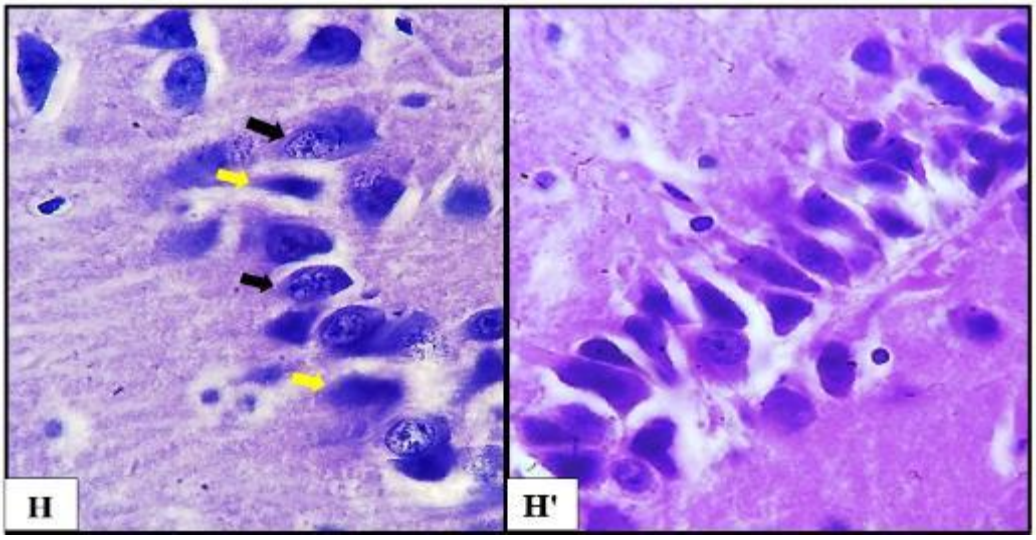

Fig. (7) Cresyl violet stained sections of the rats' hippocampus of group (0.1 mg/ kg body weight paraquat once daily for two successive months) showing **(H)** The nuclei display the uniform dispresion of hetero chromatin characteristic of neurons and have prominent nucleolus (black arrow). These large, neurons are found predominantly at hippocampus levels (CA3) in polymorphic region, the neuronal elements in the layer are somewhat minimal and stain more intensely (yallow arrow).

The layers that contain enlarged and excess astrocytes were observed Through of the CA2 there are also smaller, more darkly staining cells, the nuclei of which had irregular membranes. (fig8)

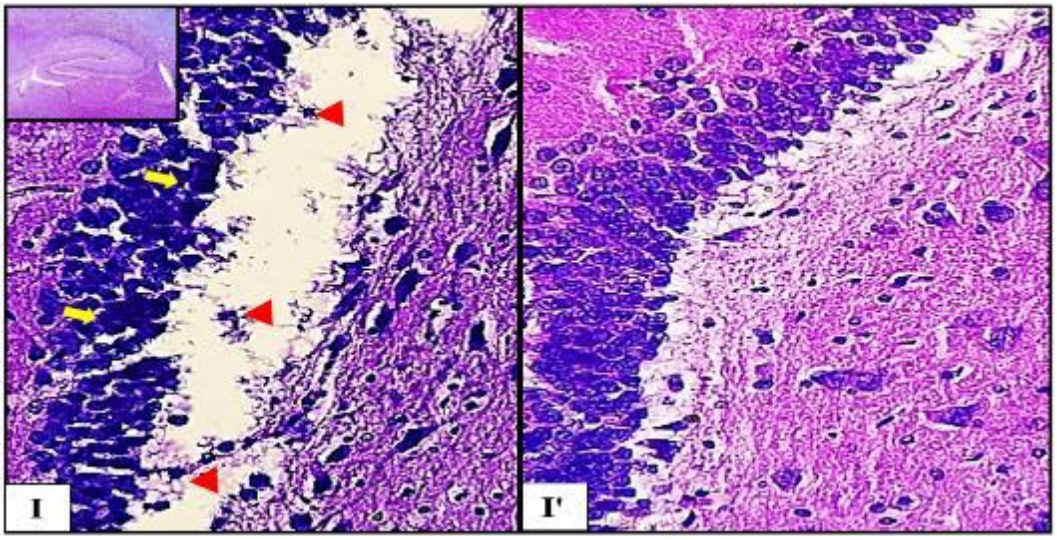

Fig. (8) Hematoxylin and Eosin stained sections of the rats' hippocampus of group (0.1 mg/ kg body weight paraquat once daily for two successive months) showing **(I)** layers contain enlarged and excess astrocytes (head arrow) Throughout the CA2 there are also smaller, more darkly staining cells, the nuclei of which have irregular membranes. These cells are probably the precursors of glial cells (yallow arrow) compared to the control **(I')** x400.

Golgi cortex method was used to detect the presence of neuron that degenerated in CA1, CA2 and CA3 regions (molecular layer, polymorphic layer and pyramidal layer) by pigmenting degenerated neurons with silver nitrate (fig 9).

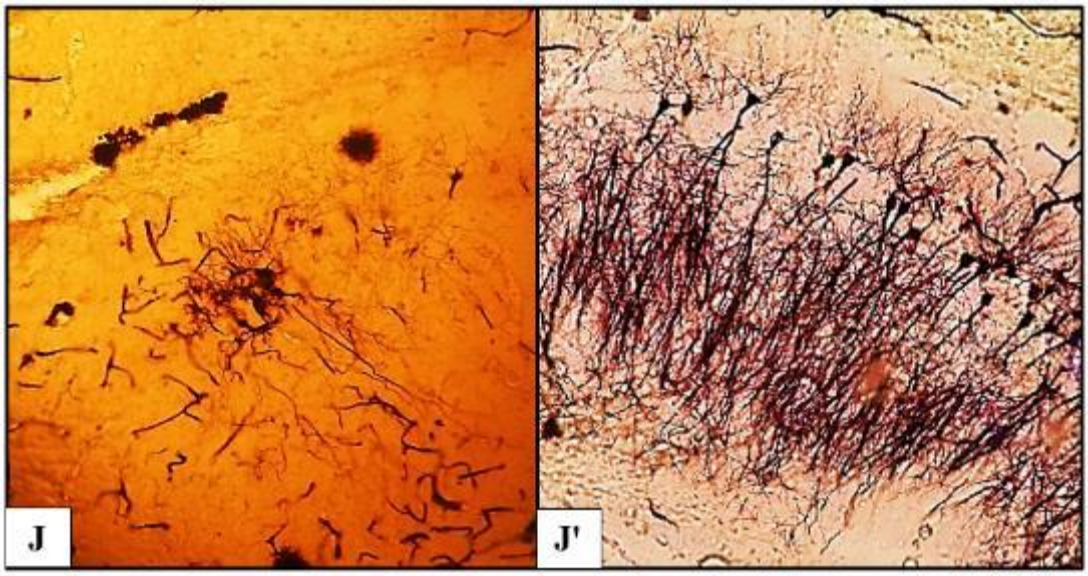

Fig. (9) Golgi cox method stained sections of the rats' hippocampus of group (0.1 mg/ kg body weight paraquat once daily for three successive months) showing **(J&J')** to detect the presence of neuron that degenerated in CA1& CA2 regions (molecular layer) x600.

We also observed the increase of astrocytes of glial cells in addition to the changes in spine density after administration with paraquat apical dendritic spine density of a CA1 subfield pyramidal neurons (fig10).

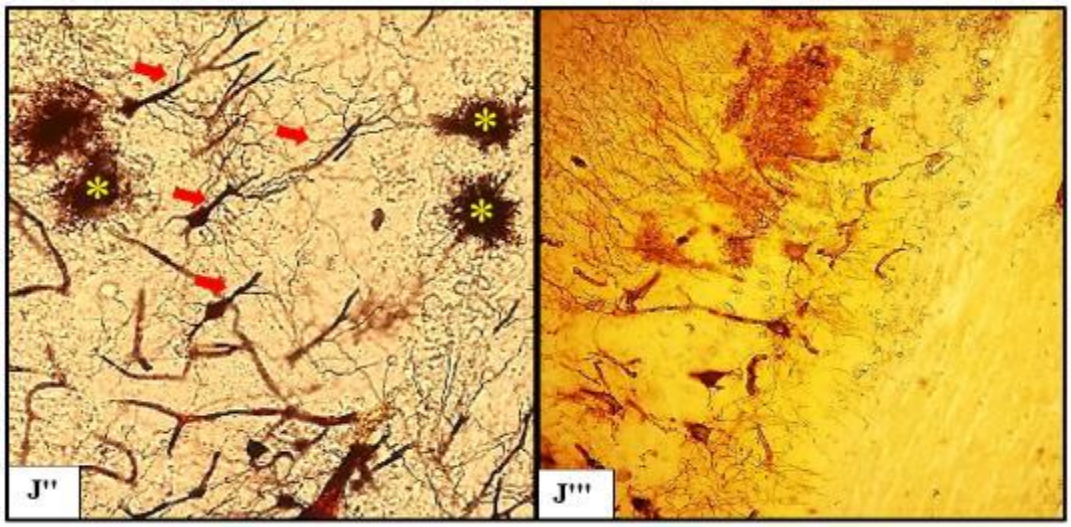

Fig. (10) Golgi cortex method of the rat Hippocampus of group (0.1 mg/ kg body weight parquet once daily for three successive months) showing **(J'')** presence of the prevailing changes in spine density after administration with paraquate apical dendritic spine density (red arrow) of a CA1 subfield pyramidal neuron and increase of astrocytes of glial cells (*), Compared to the control **(J''')**.

4. Discussion:

Despite the increasing amount of investigated in this field, the effects of repeated or paraquat exposure at low to moderate levels in both humans and animal remains unclear. This study used lower doses and longer duration of exposure, which caused a barely detectable cholinesterase inhibition, followed by cellular damage in the brain. Moreover, the study investigated the effects of paraquat on some regions in the central nervous system that are associated with PD and AD. In brief, the histological effects on the hippocampus and SN of rat brains that were treated with 0.1 mg\g of paraquat were confirmed. The different effects of oral administration of massive amounts of paraquat may be related to the rates of entry of paraquat into the central nervous system. Thus the tissue responses reflect a summation of factors such as rate of intestinal absorption, transamination within the intestinal epithelium, and rates of movement across the blood brain barrier(BBB) by amino acid transport [20]. Our cases with AD confirmed their degenerative changes in pyramidal neurons from hippocampus. In addition to neurofibrillary tangles (NFT) that develop from intracellular pre-tangles containing misfolded tau and small tau aggregates to mature NFTs containing bundles of cross-linked tau filaments before the neuron dies and an extracellular ghost tangle remains, Intracellular neurofibrillary tangles (NFTs) formed by aggregates of hyperphosphorylated tau protein [22]. and ß Amyloid plaques that commonly referred as neuritic plaques or senile plaques are extracellular desposites of ß Amyloid protein (Aß) having acentral core of Aß surrounded by numerous abnormal axons and degenerating mitochondria. The most abundant component of the plaque core is Aß a peptide of approximately 40-43 aminoacids[23]. This is a characteristic of tau protein in Alzheimer's disease and several tauopathies associated with tau unfolding, α-synuclein in Parkinson's disease, Usually, the self-aggregation products are toxic to these cells, and toxicity spreads all over different brain areas. these events occur as a result of neuroinflammatory cascades involving alterations in the cross-talks between glial cells and neurons as a consequence of the activation of microglia and astrocytes. These along with the oligomers of β-amyloid (Aβ) peptide became the major hallmarks of this disease. NFT start forming because of the increase in p-tau. It is also interesting that almost 60% of AD subjects present LB, which may cause wide degeneration in different regions in the brain and changed the size to be smaller by blurring most of the features of their brain [16]. In Parkinson's and Alzheimer's disease, the proteins that primarily aggregate are α-synuclein, Tau and β-amyloid proteins, respectively, although the latter are also found in Parkinson's disease. Insoluble α-synuclein fibrils make up the Lewy bodies and Lewy neurites. These are predominantly present in the pigmented neurons of the substantia nigra and in other neuronal populations at the peripheral and central levels [9]. Other studies suggested that the etiology of PD is a genetic basis that interacts with environmental factors. In conjunction with previous studies, this study found apoptosis, which is programmed cell death, in the hippocampus, neuronal loss in the compacta pars of the SN, and dopaminergic degeneration due to chronic treatment with other types of pesticides [11, 28]. Moreover, idiopathic PD was characterized by the

progressive loss of dopaminergic neurons in the SN (pars compacta), which leads to dopamine depletion [9]. This improved after the administration of these compounds, as demonstrated in the current research. Several studies reported a possible correlation between exposure to pesticides and the development of neurodegenerative disorders [16, 29]. Since paraquat is the most used pesticides, the previously mentioned studies focused on a link between paraquat and the most common neurodegenerative diseases, PD and AD. Interestingly, some types of herbicides have been proved to induce degeneration in the dopaminergic system [3, 5]. Inflammation by pesticides, such as paraquat, is thought to be the primary pathophysiological reason for neuronal degeneration, which occurs when the neutral amino acid transporter mediates the Na+ dependent entry of organophosphate into dopaminergic neurons. Consequently, organophosphate impairs redox recycling and induces oxidative stress, which leads to neuronal death [4, 14, 27]. The increased permeability of the blood-brain barrier (BBB) and neurovascular dysfunction have been associated with severe conditions of PD. This effect could be linked to the infiltration of inflammation molecules that cause microglia activation and dopaminergic neurons death [24]. Numerous studies have demonstrated that exposure to a variety of paraquat at lower and prolonged levels induces major changes in the central nervous system, such as changes to the gene expression, cell signaling pathways, and cellular ultrastructure. Moreover, exposure to various pesticides increases the strict inclusion of many glial cells [30]. It is worth mentioning that microglia are the resident immune cells (macrophages) of the brain, which can be triggered and activated in response to pro-inflammatory triggers or neuronal death. In this case, several reactive oxygen species and pro-inflammatory factors (e.g., tumor necrosis factor α and interleukin-1β) are produced, which contribute to neurotoxicity and degeneration. Similarly, pesticides that include malathion may cause an inflammatory response, which could lead to the activation of microglia, as found by several studies [1]. Since the metabolism of astrocytes is an in dissociable link between neuronal health and synaptic functions, it is important to be mindful of how environmental toxicants can impact human health. Furthermore, environmental toxicant exposure can result in glucose dysfunction, including the involvement of the GLUT1 transporter, especially the astrocyte-specific transporter[13]. Within the CNS, there is an overlap between the peripheral and astrocytic mechanisms. The engagement of the CYP detoxification system, which is robustly expressed in astrocytes as part of its role as the primary defense against xenobiotic penetrance into the CNS[14], suggests that astrocytes are crucial for a system-wide response to toxicants [18, 31]. The current study shows an increase of the amyloid fibers that were stained in Congo red in the spaces between neurons. The deposition of Aβ (mainly Aβ 40 and 42) caused them to form amyloid plaques, which are associated with reactive gliosis [25]**.** In these areas, the activated microglia are recruited, and the reactive astrocytes exhibit a morph-functional remodeling, which modifies their interactions with neurons. Despite the reasonably close correlation between the increased phosphorylation and neuron degeneration, this study concludes that exposure to pesticides compounds degenerates some neurons in the polymorphic and pyramidal layers. This was

proven by various staining methods. According to the hypothetical basis of binding silver to disintegrate products of proteins in neurons, this study demonstrates the emergence of necrosis in multiple spaces among the neurons, with increased phosphorylation of the proteins in most of the neurons' membranes. Silver-forming complexes with individual amino acids and progressive fragmentation of proteins (proteolysis) in disintegrating neurons lead to expansive sites for silver to form complexe [2].

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