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Histopathology of Brain Tissues of Male Rats in Experimentally Induced Oxidative Stress and the Protective Aspects of Apigenin

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ABSTRACT

Despite of the beneficial physiological role of low or moderate concentrations of free radicals at cellular levels, its harmful effects in the onset and or progression of several diseases have been documented by many workers.

Hydrogen peroxide used as an experimental source of highly reactive hydroxyl radical and it is considered the main oxidative stress inducer. In the current study the brain`s histological changes of male rats exposed to 0.75 % H_2O_2 in the drinking water and the impact of apigenin as protective agent have been investigated.

Histological sections of the brain post 4 and 8 weeks of H_2O_2 treatment showed marked degeneration and necrosis of Purkinje cells that characterized by eosinophilic cytoplasm and absence of the nuclei. Congestion and perivascular cuffing of inflammatory cells mainly neutrophils were also seen in the pia mater compared to the animals of negative control and bultylated hydroxytoloene (BHT) treated groups.

Interestingly, histological results demonstrated less or no significant lesions in H_2O_2 treated groups administrated apigenin in both forms aglycone and glycosidic forms suggesting the potential neuro-protective effect of apigenin as antioxidant with a clear priority to the apigenin in aglycone form. Moreover, these results may provide promising therapy against oxidative stress complications such as brain damage.

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1- INTRODUCTION

Oxidative stress and inflammatory response are the main factors contributing in neurons functional deterioration (balance, movement and breathing) brain tumors, cerebral ischemia and neurodegenerative diseases; such as Alzheimer, Parkinson; Huntington, and multiple sclerosis [1, 2, 3, 4, 5].

Neurons become more vulnerable to oxidative stress due to their high level of metabolic activates high oxygen demand, endogenous antioxidant, and rich levels of unsaturated fatty acids in the brain lipids [6, 7].

Hydrogen peroxide as an important endogenous reactive oxygen species implicated in health and disease [8], produced in B- amyloid aggregation, dopamine oxidation as well as brain ischemia reperfusion [9]. Moreover, H_2O_2 has shown to initiate cellular damage via either direct protein oxidation DNA, and lipids or acting as signaling molecules to elicit apoptotic pathway [10]. However, lower level of endogenous antioxidant in the brain has been encouraged high metabolic performance, oxygen requirements, and low antioxidant defense capability of neurons in the brain [11].

Apigenin, as a natural flavonoid present in fruits, vegetables and further plant materials [12]. Several studies demonstrated that apigenin has antioxidant [13], anti-inflammatory [14] with neuroprotective efficacy [15], In addition to regulatory potency in cellular energy homeostasis and immune cells gene expression [16].

In the current study, we assessed the ability of apigenin in both forms, aglycosidic and aglycone forms, to ameliorate the oxidative stress in brain tissue of rats induced by exogenous hydrogen peroxide in drinking water in comparison with butylated hydroxytolouene as standard antioxidants.

2- MATERIAL AND METHOD

The seeds of parsley (*Petroselenium sativum*) was obtained from local markets in Baghdad- Iraq, and documented at the National Herbarium- Iraqi Botany Directorate. Following the procedure of Harborn [17] with some modifications suggested by Al-Kawary [18], extraction of apigenin from crushed parsley seeds in aglycone form was conducted, while the apigenin in glycoside form was prepared according to method of Ikhan [19].

Identification of both forms of apigenin (aglycone and glycoside) was carried out on silica gel type G thin layer plates and the following organic solvents such as acetic acid, ethyl- acetate and toloune at a ratio 5: 12: 36 was utilized as a mobile phase [20]. Ultra violet detector at wavelength 254 nm was employed to explore separated spots. Standard apigenin from Xi`an- China was used as a reference apigenin.

Five experimental groups (adult male Albino Wister rats 250-300 gm) were utilized (n=10 each group). Negative control group (C) was allowed to *adlibitum* supply of drinking water while the rest of the animals were divided as follows:

Group T1: Positive control group, animals exposed to H_2O_2 (0.75%) in drinking water.

Group T2: animals exposed to H_2O_2 (0.75%) in drinking water plus daily oral administration of butylated hydroxyl toloune at 25 mg/kg body weight.

Group T3 and T4 animals exposed to 0.75% hydrogen peroxide plus daily administration of 150 mg/kg/B.W of apigenin in glycosidic and aglycone forms, respectively.

Dissection Of The Rats Brain

After 4 and 8 weeks of experimental period, animals (n=5) from each group were sacrificed and brain of each rat was separated from the surrounding tissue and gently rinsed with a 0.9% NaCl and immediately fixed in 10 % neutral- buffered formalin (NBF) for 72hr. The fixed specimens were trimmed, washed and dehydrated in ascending grades of ethanol. After that, specimens were cleared in xylene, embedded in paraffin wax, sectioned at 5 mm and stained with hematoxylin and eosin (H& E) for microscopic examination [21, 22].

3- RESULTS AND DISCUSSION

Histological Examination of Brain H_2O_2 Treated Male Rats

The microscopic examination of brain tissues after four weeks of experiment showed areas of hemorrhage with inflammatory cells infiltration in pia mater [fig. 3]. Moreover, edema around the blood vessels and neurons in the brain parenchyma were recorded as compared to the control group [fig 1 and 2].

Whereas, eight weeks post-H_2O_2 treatments the main changes in the brain tissue were necrosis of Purkinje cells that manifested by rounded eosinophilic cytoplasm with acentric or no nuclei [fig. 4]. Inflammatory cells mainly

neutrophils were seen within the congested blood vessels and pia mater with perivascular in the brain parenchyma [fig. 5, 6].

Hydrogen peroxide as stress inducer of ROS, that caused a various cell injury and the high levels of exogenous of H_2O_2 in this study probably may overcome cellular antioxidant defenses that led to cytotoxicity and cellular dysfunction [23, 24, 25]. Exposure of neurons to an excess exogenous oxidant caused both apoptosis and necrosis [26, 27].

An excess H_2O_2 may induce endothelial dysfunction with subsequent elevation in phosphorylation of tyrosine kinesis and alteration of vessel permeability [28, 29]. Moreover, production of transcription factors; NF-IB and activator protein 1 (AP-1), which participate in the expression of different adhesion molecules (VCAM-1, ICAM-1, E-select in and other cytokines) may other mechanisms through which H_2O_2 exert issue damage [30].

Also, the reaction between H_2O_2 and Fe^{+2} [fenton reaction] generates highly reactive hydroxyl radicals ($OH^{\cdot}$) and is considered to be the principle pathway for oxidative damage [31].

The result of this study confirmed the potential capability of apigenin in recovering the deleterious effect of H_2O_2 and lesser neurotoxicity at the cellular levels after four weeks of treatment with apigenin in glycosidic [Fig. 9 and 10], and aglycone forms [Fig. 11]. In comparison to BHT [Fig. 7 and 8]. Treated animals.Tthe protective role of apigenin in both forms was more obvious post eight weeks of the treatments [Fig. 13, 14] compared to BHT group [Fig. 12]. The protective role of apigenin may attribute to its role in down regulating TLR4/NF-KB signaling pathways, lowering the concentration of IL-6 level and TNF-X, and inhibiting the mitochondria- mediated neuron apoptosis, as well as attenuating the expression of Cox-2 and iNOS at cellular level [32, 33].

Apigenin neuroprotective role against oxygen and glucose deprivation (OGDIR) injury in rats hippocampal neurons may associate with its ability to improve sodium pump activity [34].

Apigenin`s neuroprotective activity along with other flavonoids against oxidative damage induced by H_2O_2 in a PC12 cells may associated with an elevation in glutathione reductase, catalase and glutathione peroxidase levels in scavenging H_2O_2, inhibitor of membrane, DNA damage, as well as caspase-3 activity and regulation of calcium ion concentration also, mitochondrial membrane potential maintenance [35].

Moreover, the role of apigenin as a vital neuro-immune modulatory agent in the treatment of neurodegenerative conditions and improvement of various states of cognitive dysfunction, have been considered by many workers [36].

5- CONCLUSION

The results of histopathological changes of the brain tissues in experimentally- induced brain damage by oxidative stress in rats clarified obviously the impact of apigenin (glycosidic and aglycone forms) in ameliorating the deleterious effect of hydrogen peroxide as oxidizing agents manifested by minimizing to a great extent the neurotoxicity at the cellular level confirming the neuro- protective role of apigenin as promising antioxidant.

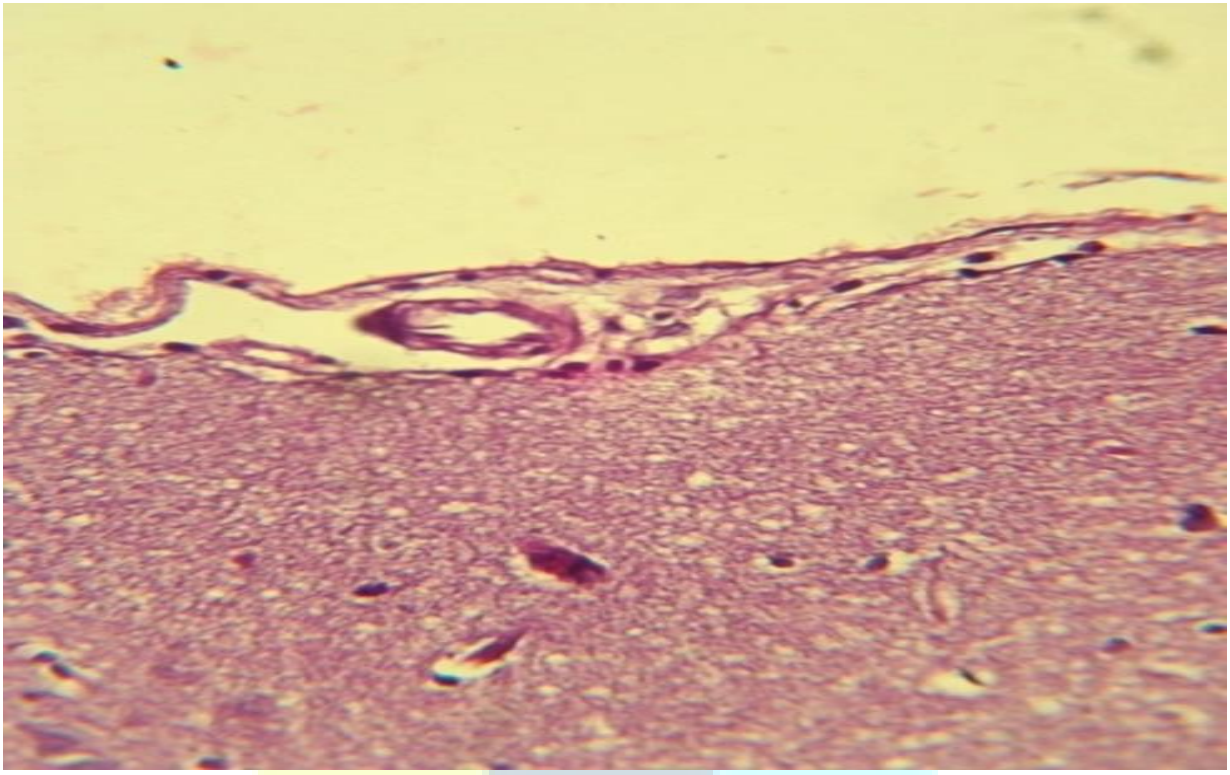

Fig (1): Photomicrograph of the brain section of negative control rat shows normal cerebral cortex and pia matter (Black arrow) (H and E stain, ×40).

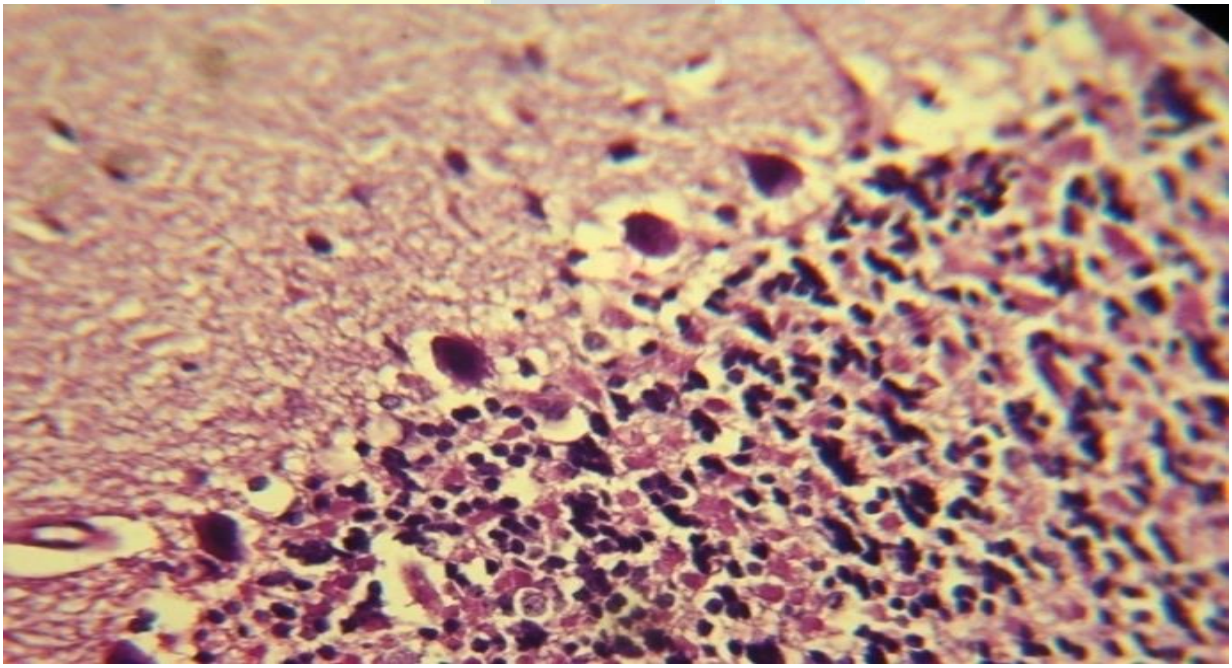

Fig (2): Photomicrograph of the brain section of negative control rats shows normal Purkinje cells (Black arrow) and granular layers of the cerebellum (Red arrow) (H and E stain, ×40).

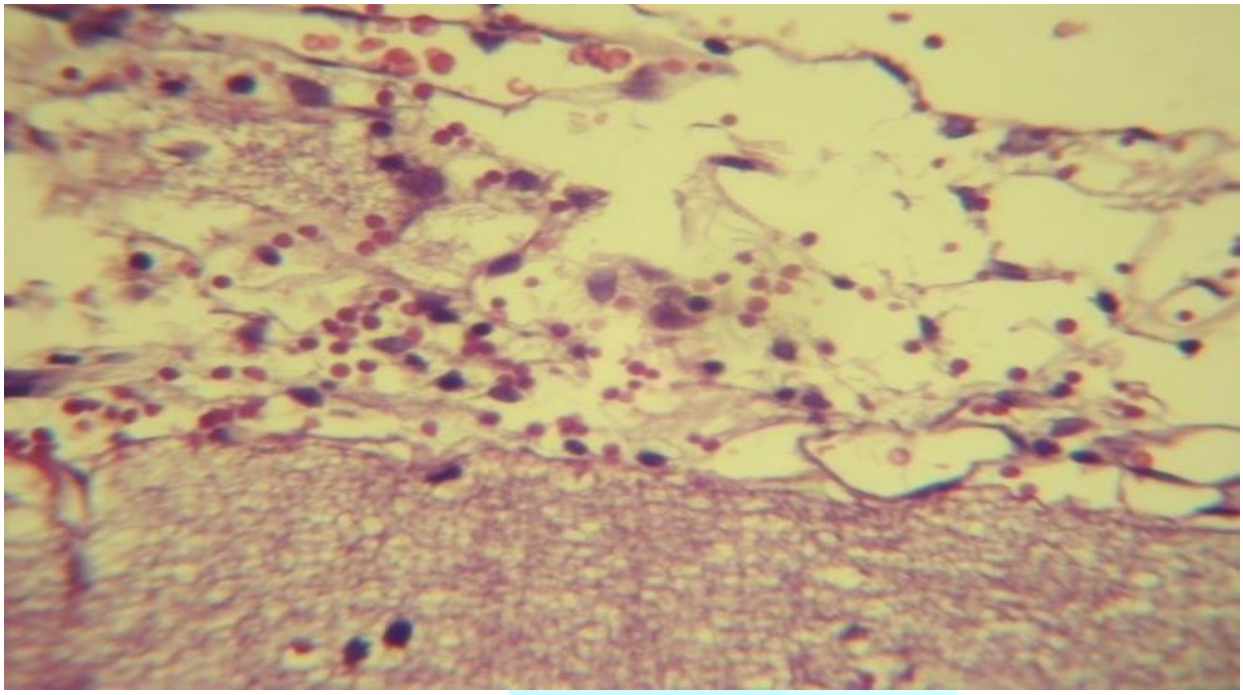

Fig (3): Photomicrograph of the brain section of rat after 4 weeks of treatment with 0.75% of H_2O_2 in drinking water, shows hemorrhage (Red arrow) with inflammatory cells infiltration in pia mater (Black arrow) (H and E stain, ×40).

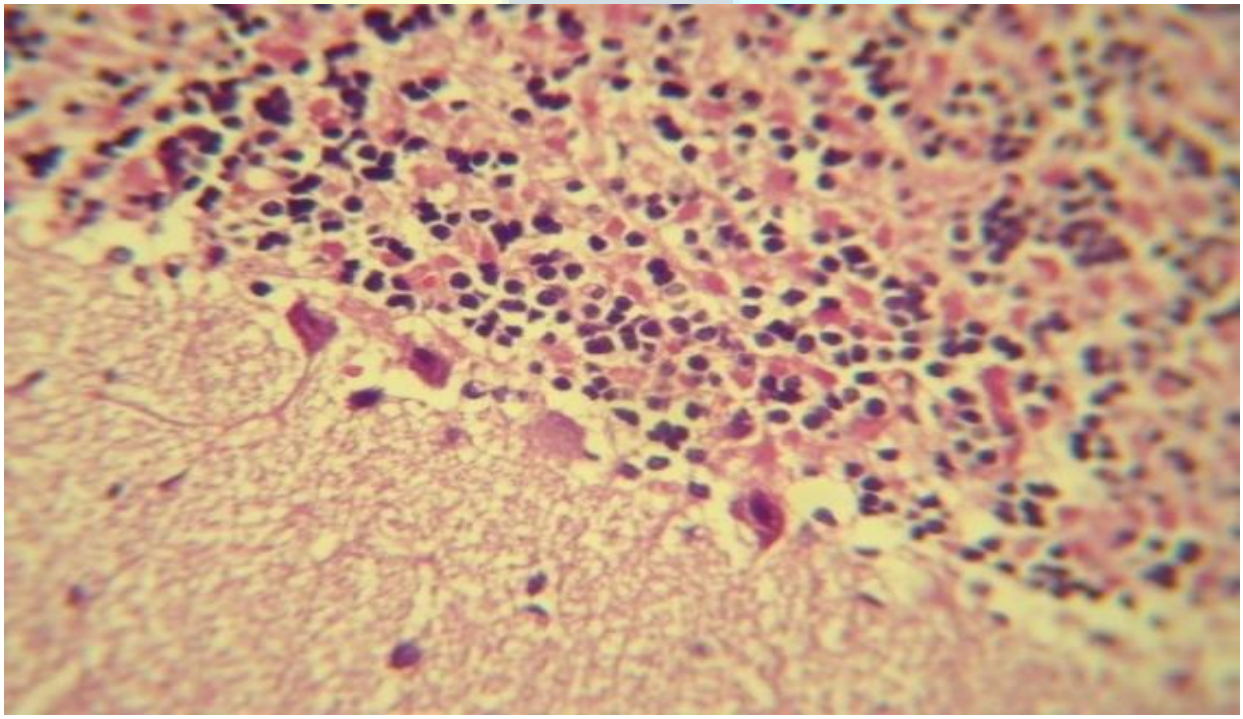

Fig (4): Photomicrograph of the brain section of rat after 8 weeks of treatment with H2O2 0.75% in drinking water, shows necrotic Purkinje cells with eosinophilic cytoplasm with no nuclei (Red arrow) (H and E stain, ×40).

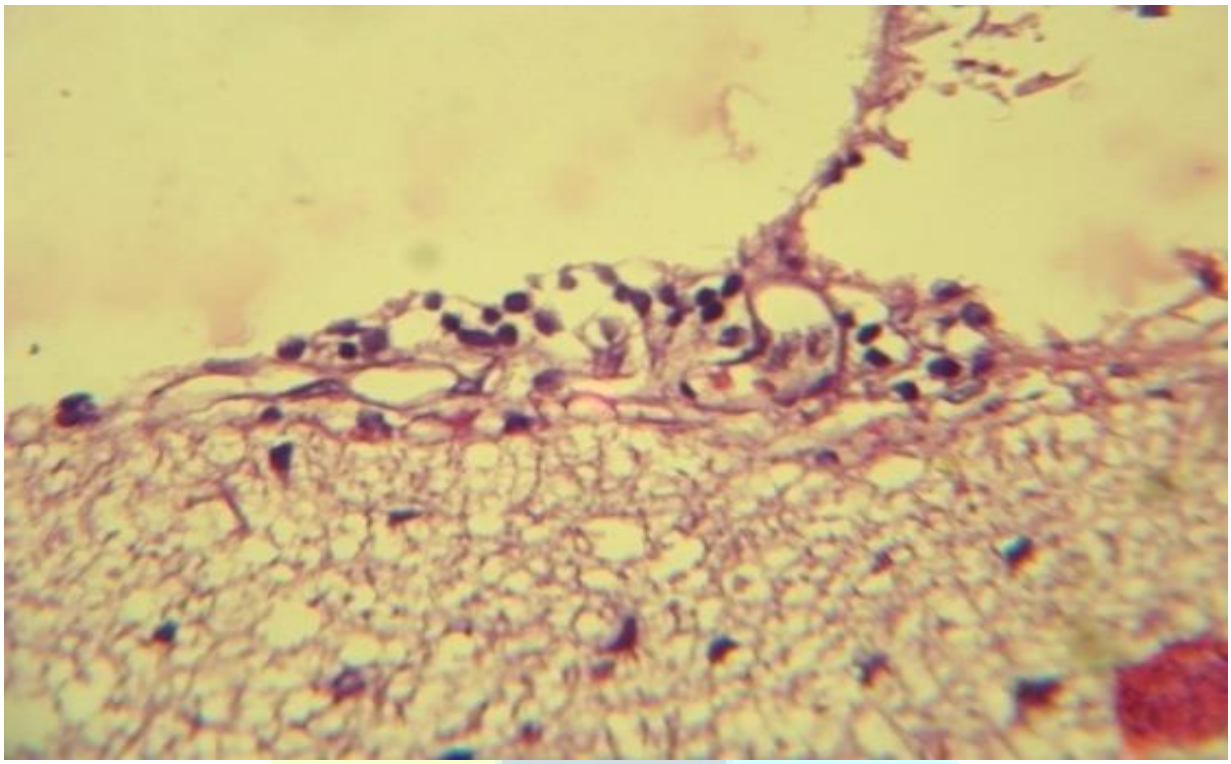

Fig (5): Photomicrograph of the brain section of rat after 8 weeks of treatment with H_2O_2 0.75% in drinking water, shows inflammatory cells in the pia mater (Black arrow) (H and E stain, ×40).

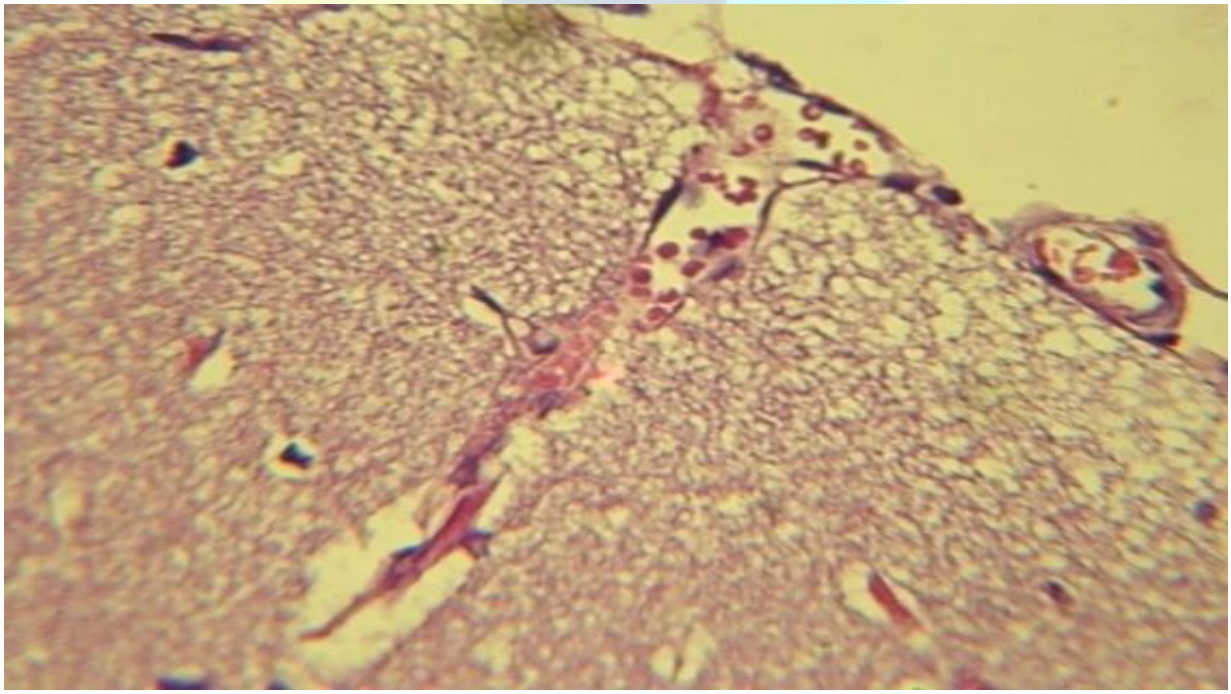

Fig (6): Photomicrograph of the brain section of rat after 8 weeks of treatment with H_2O_2 0.75% in drinking water, shows congested blood vessels with neutrophils (Black arrow) in their lumen and perivascular edema (Red arrow) (H and E stain, ×40).

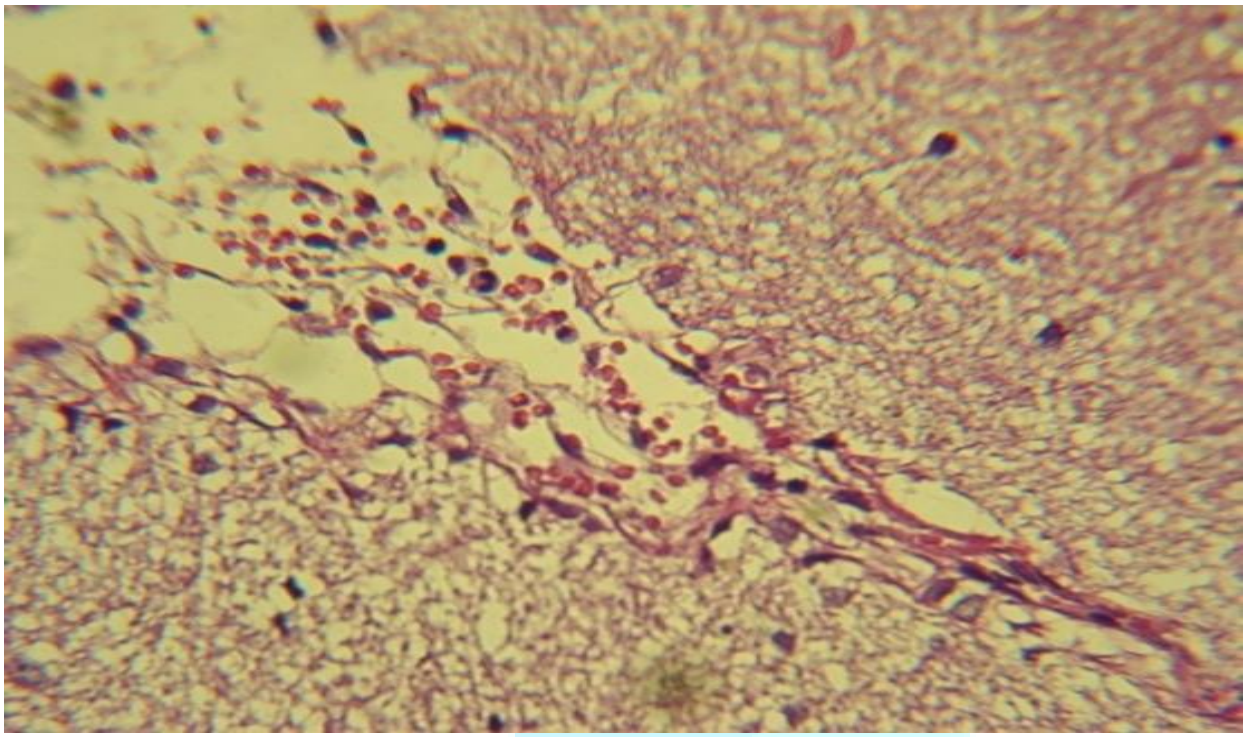

Fig (7): Photomicrograph of the brain section of rat after 4 weeks of treatment with 0.75% H_2O_2 and oral administration of BHT 25mg/kg of B.W. shows congested blood vessels with neutrophils in pia mater (Black arrow) (H and E stain, ×40).

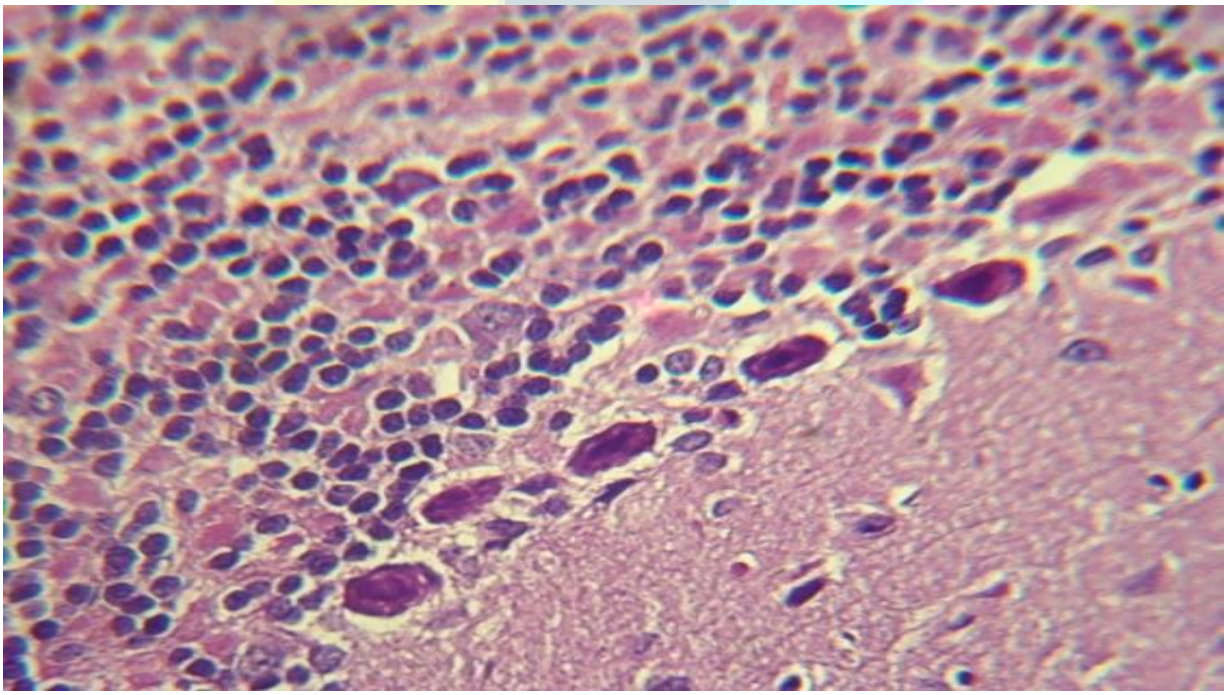

Fig (8): Photomicrograph of the brain section of rat after 4 weeks of treatment with 0.75% H_2O_2 and oral administration of BHT 25mg/kg of B.W. shows no clear lesion in the Purkinje cells (Black arrow). (H and E stain × 40).

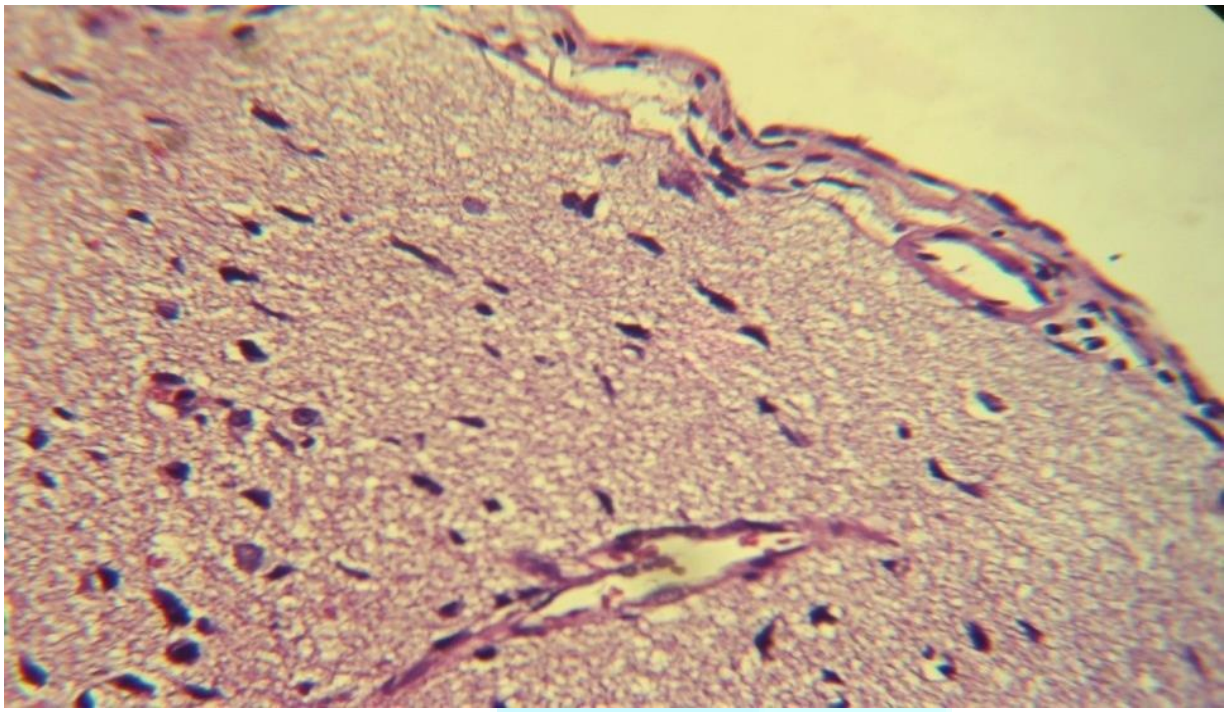

Fig (9): Photomicrograph of the brain section of rat after 4 weeks of treatment with 0.75% H_2O_2 and oral administration of apigenin glycoside 150mg/kg of B.W. shows no clear lesion in the cerebral cortex. (H and E stain, ×40).

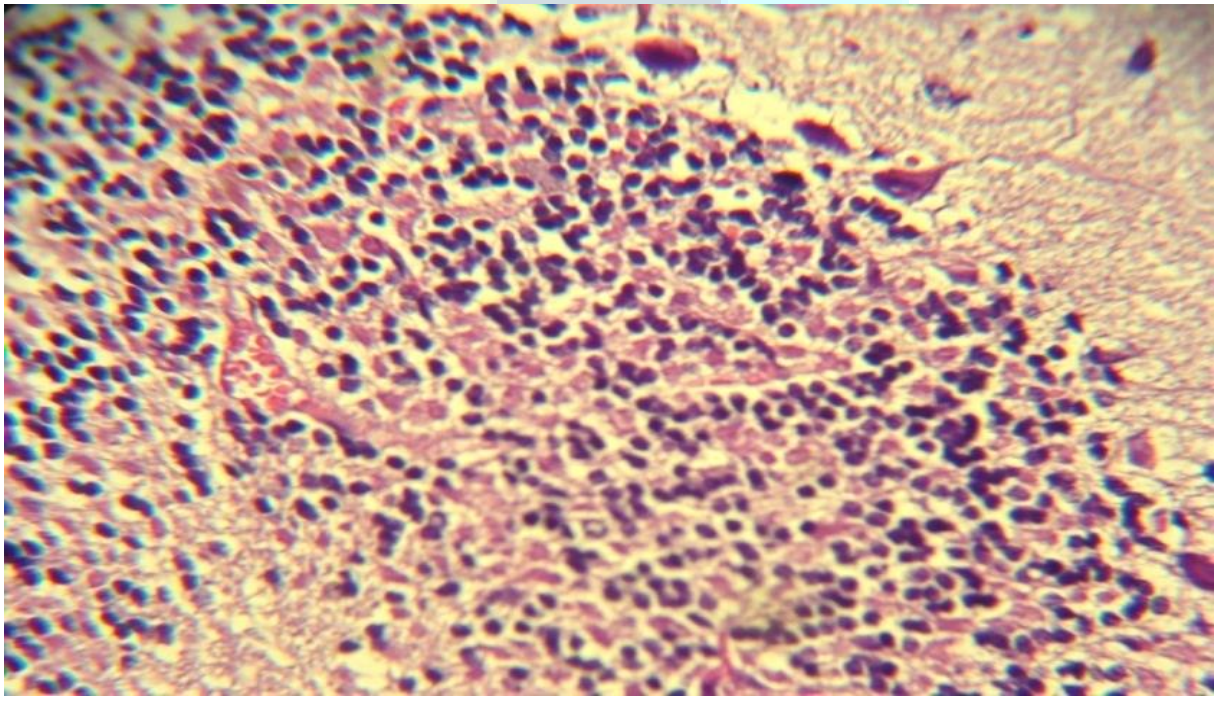

Fig (10): Photomicrograph of the brain section of rat after 4 weeks of treatment with 0.75% H_2O_2 and oral administration of apigenin glycoside 150mg/kg of B.W. shows no clear lesion in the Purkinje cells (Black arrow). (H and E stain × 40).

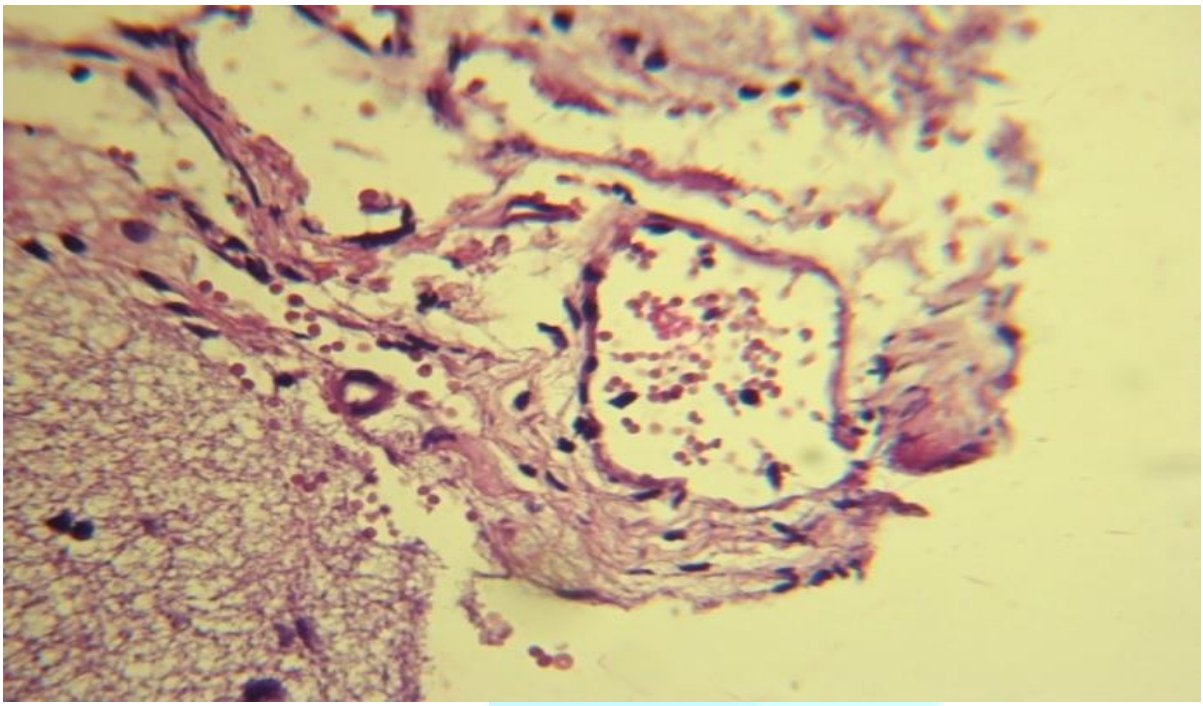

Fig (11): Photomicrograph of the brain section of rat after 4 weeks of treatment with 0.75% H_2O_2 and oral administration of apigenin aglycone 150mg/kg of B.W. shows no clear lesion. (H and E stain × 40).

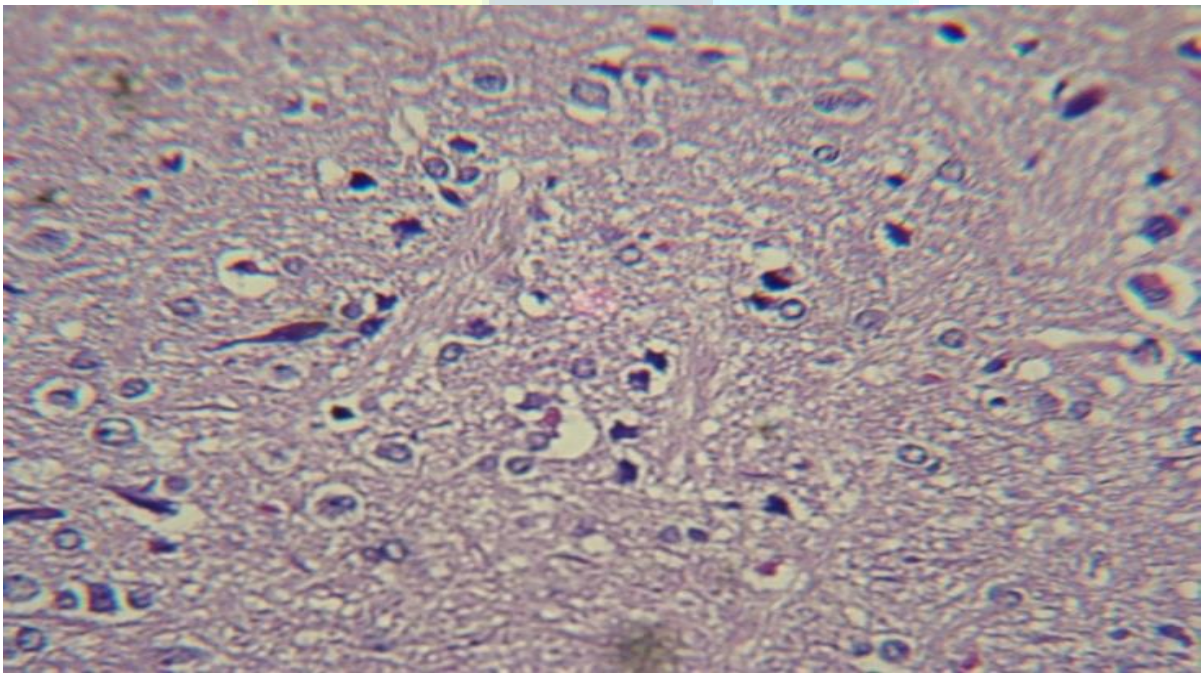

Fig (12): Photomicrograph of the brain section of rat after 8 weeks of treatment with 0.75% H_2O_2 and oral administration of BHT 25mg/kg of B.W. shows no clear lesion (H and E stain, ×40).

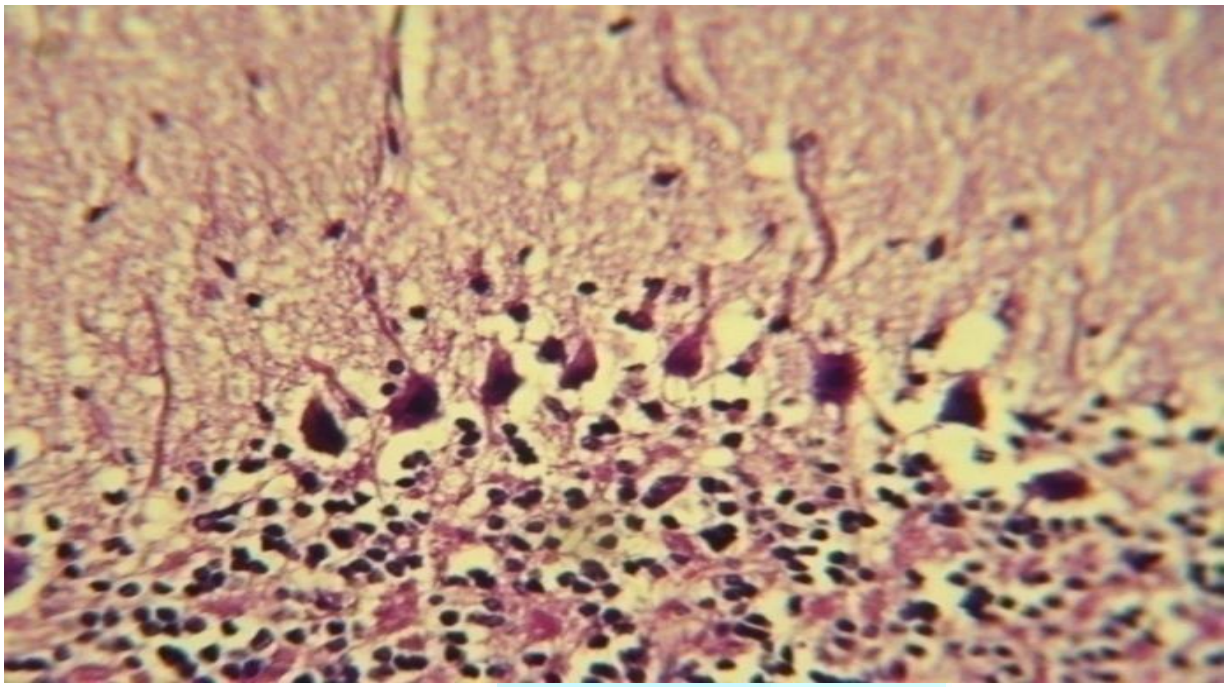

Fig (13): Photomicrograph of the brain section of rat after 8 weeks of treatment with 0.75% H_2O_2 and oral administration of apigenin glycoside 150mg/kg of B.W. shows no clear lesion in the Purkinje cells (Black arrow). (H and E stain × 40).

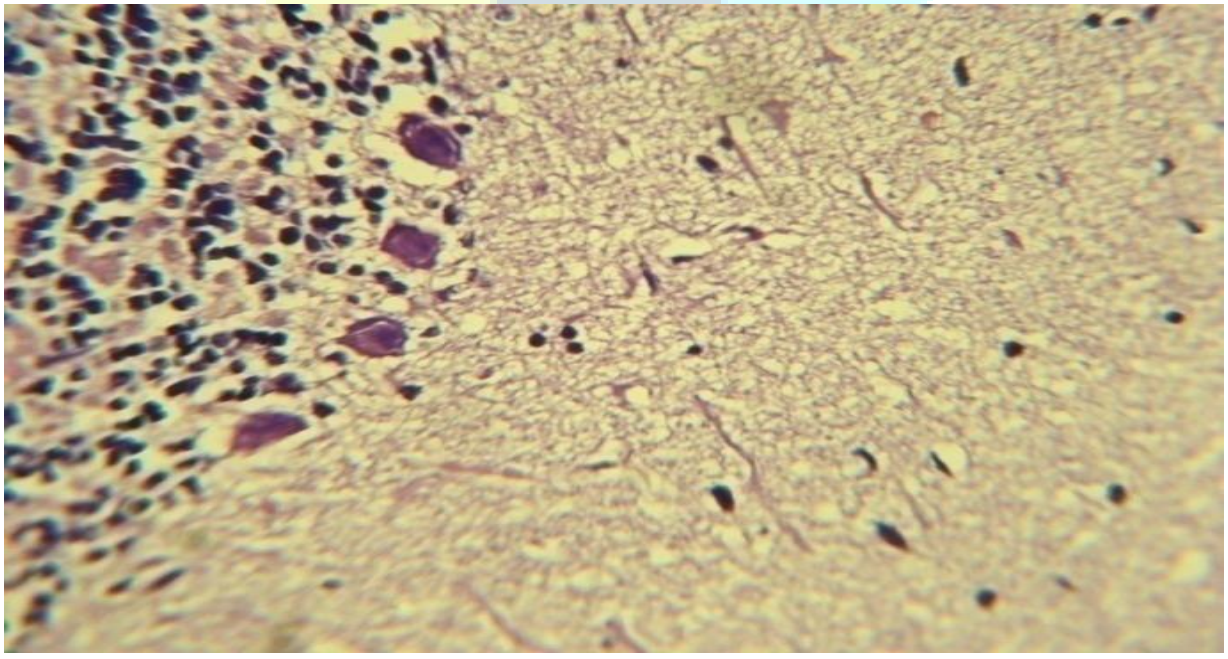

Fig (14): Photomicrograph of the brain section of rat after 8 weeks of treatment with 0.75% H_2O_2 and oral administration of apigenin aglycone 150mg/kg of B.W. shows no clear lesion in the Purkinje cells (Black arrow). (H and E stain × 40).

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التشريح المرضي لأنسجة دماغ ذكور الجرذان المعرضة تجريبيا للاجهاد التأكسدي والدور الوقائي للأبجنين

الخلاصة

على الرغم من الدور الفسيولوجي المفيد للتراكيز القليلة والمتوسطة للشوارد الحرة على المستوى الخلوي ولكن تأثيره الضار في بداية نشوء المرض او تطوره اللاحق تمت الاشارة اليه من قبل عدد كبير من الباحثين.

استخدم بيروكسيد الهيدروجين مصدراً تجريبياً لجذور المجاميع الحرة الشديدة الفعالية والذي يعد عاملاً حيوياً في الاجهاد التأكسدي المستحث او المحفز، ومما تقدم فقد اجريت هذه الدراسة للوقوف على التغييرات الحاصلة في انسجة دماغ ذكور الجرذان المعرضة لبيروكسيد الهيدروجين بتركيز ٠,٧٥% في ماء الشرب ودور الابجنين المستخلص من بذور البقدونس بصيغتيه الكلايكوسيدي [أرتباط مجموعة غير كربوهيدراتية مثل الكحولات او الفينولات مع جزيئة السكر] والجزء الاكلايكوني [الجزء غير السكري] وبجرعة ١٥٠ ملغم/ كغم من وزن الجسم مقارنةً بمجموعتي السيطرة والمجرعة بوتيل هيدروكسي تولوين ٢٥ ملغم / كغم من وزن الجسم.

أظهرت نتائج المقاطع النسجية لدماغ الجرذان المعرضة تجريبيا للأجهاد التأكسدي مقارنةً بالمجاميع الاخرى بعد اربعة وثمانية اسابيع من معاملتها يومياً ببيروكسيد الهيدروجين حصول تلف ونخر واضحين في خلايا بركنجي والذي تميز بميل السايتوبلازم للصبغة الحامضية وغياب النواة، كما لوحظ أيضاً وجود احتقان وكتل من الخلايا الالتهابية تحيط بأوعية العدلات الدموية والغشاء المغلف للمخ والام الحنون.

ومن المثير للاهتمام أن المقاطع النسجية المأخوذة من المجاميع المعرضة للأجهاد التأكسدي والمجرعة بالابجنين أظهرت وجود تلف والتهاب بسيط جداً او غيابهما مما يؤكد الدور الوقائي للابجنين (بصيغتيه مع أولوية واضحة لصيغة الاكلايكون) وقدرته كمادة مانعة للأكسدة للحد من الاجهاد التأكسدي ومضاعفاته المؤثرة على أنسجة الدماغ.