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Research Article

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Vitamin C Effect on Dementia Induced by Scopolamine Hbr in Mice (Histological Study)

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ABSTRACT

Dementia is a deterioration in cognitive ability, which affects memory, and behavioral and psychological symptoms. Scopolamine hydrobromide is a muscarinic receptor antagonist that induces memory impairment and oxidative stress. Vitamin C is a strong antioxidant agent; its deficiency may be associated with an increased risk of dementia. This work aims to find out the effect of vitamins C on behavior in dementia induced in mice using scopolamine hydrobromide. The brain histological effect of vitamin C on dementia induced by scopolamine hydrobromide in albino mice is studied. Method: Male albino mice were divided into ten groups of six each. Group 1: Administered T80(1%) for one week, Group 2: Administered scopolamine HBr at a dose of 6 mg/kg for one week, Group 3: Administered vitamin C at a dose of 500mg/kg for one week, Group 4: Administered scopolamine HBr plus vitamin C for one week, Group 5: Administered scopolamine HBr for one week, followed by vitamin C administration for another one week, Group 6: administered scopolamine plus Donepezil for one week.

Drugs were administered by intraperitoneal route, at the volume of 5ml/kg body weight. All drugs were freshly prepared; mice were killed by neck dislocation; the brains were immediately removed, inserted in 10% formalin, and sent to the histology department, for brain histological study. Results and conclusion: Dementia model induced by scopolamine HBr, showed mild mononuclear cellular (lymphocytic) infiltration and aggregation, mild vascular proliferation, and perivascular edema. In the cerebral cortex, focal gliosis with microglial proliferation and distributed astrocytes was observed. Some shrunken neurons exhibited eosinophilic cytoplasm and shrinkage of basophilic nuclei. The hippocampus was unremarkable. Brain histological studies showed oxidative changes in vitamin C treated group compared to the control; vitamin C may act as a pro-oxidant. The combined treatment of vitamin C and scopolamine HBr produce more damage to cerebral cortex and hippocampus. The damage was severe when vitamin C is administered after scopolamine HBr.

Key Words- *Dementia; Scopolamine hydrobromide;l Vitamin C.*

INTRODUCTION

Dementia is a chronic syndrome of progressive deterioration in cognitive ability, and behavioral and psychological symptoms (BPSD).[1,2] Treatments have been approved for the dementia stage of the disease and provide only symptomatic benefit but no slowing of progression.[3] There is no cure yet for dementia, more than 250 clinical trials have been conducted on a variety of treatments, and all have failed to reverse the progression of the disease.[4]

Acetylcholinesterase inhibitors (such as donepezil) are used for all stages of dementia[5] and NMDA antagonists (memantine) are used for moderate to severe dementia.[6] However, acetylcholinesterase inhibitors can only improve cognitive symptoms of dementia for a certain period but cannot modify the disease course;[7] also, neuropsychiatric symptoms associated with dementia need to be treated.

Scopolamine HBr is used in nausea and motion sickness management.[8] Scopolamine HBr can permeate the blood-brain barrier and is widely used in rodent model of dementia.[9] The cognitive impairment properties of scopolamine HBr contributed to its application in animal neurocognitive research.[10] Scopolamine HBr induces dementia by blocking cholinergic transmission in the hippocampus and prefrontal cortex.[11,12]

Vitamin C is a water-soluble vitamin; it has anti-inflammatory and strong antioxidant effects by limiting the damaging effects of free radicals.[13] Vitamin C acts as a donor of single hydrogen atoms, and the radical anion

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monodehydroascorbate reacts mainly with radicals. These properties account for the remarkable antioxidant actions of vitamin C.[14] Vitamin C may reduce the cognitive deficits induced by dementia.

Therefore, the main objective of this research is to find out the effect of vitamin C on dementia induced by scopolamine hydrobromide using albino mice.

Materials and Methods

Animals: Male albino mice (25-40g) were obtained from the animal house of the Faculty of Pharmacy, University of Tripoli, they were housed in six per cage, in a room under controlled temperature (20 ± °C), and on 12/12 h light/dark cycle, with food and water available ad libitum. The animals were kept in the laboratory at least 24 hours before experiments, to adapt to the environment.

Drugs and chemicals: Scopolamine hydrobromide was obtained from BDH Chemicals Ltd-Poole-England, it was administered at a dose of 6mg/kg (chosen from pilot study). Vitamin C was obtained from BDH Chemicals Ltd-Poole-England, it was administered at a dose of 500mg/kg.[15,16] Donepezil was obtained from Milpharm Limited/Aurobind Pharm-Malta., it was administered at a dose of 1mg/kg.[17] Diazepam was obtained from Roche, Switzerland, it was administered at a dose of 1mg/kg.[18] Imipramine hydrochloride was obtained from Novartis-Spain, and it was administered at a dose of 10mg/kg.[19] Tween 80 (1%) was obtained from Riedel-De Haen AG Seelzf-Hannover, it was injected in volume 5ml/kg and used as a solvent to all drugs. Drugs were administered intraperitoneal (i.p) route, at volume of 5ml/kg body weight. All drugs were freshly prepared.

Design of the work

The study used male albino mice; mice were classified into six in each. Group 1, administered T80 (1%) for one week. Group 2, was administered scopolamine HBr (6mg/kg) for one week. Group 3, administered vitamin C at a dose of 500mg/kg/day[15,16] for one week. Group 4, administered scopolamine HBr plus vitamin C for one week (prophylaxis effect of vitamin C). Group 5, administered scopolamine HBr for one week, followed by vitamin C administration for another week (treatment). Group 6: administered scopolamine plus Donepezil for one week.

At the end of the administration, mice were killed by neck dislocation; the brains were immediately removed, inserted in 10% formalin, and sent to the histology department in medical school, for histological investigation.

The methodology and handling of animals were according to the guidelines of animal use of the Department of Pharmacology and Clinical Pharmacy, University of Tripoli.

Histological study

Brains of both control and treated male albino mice groups were collected and then fixed in 10% formalin for 24 hours. The specimens were washed twice with 70% alcohol. The fixed tissues were dehydrated in an ascending series of alcohol ranging from 70% to 100% (absolute). The dehydrated tissues were cleared with two changes of xylene (clearing agent) for 1 hour each. The pieces were then embedded in three changes of molten paraffin wax at a melting point of 60°C, 1 hour each. Then, the pieces were embedded in paraffin blocks. Brains were sectioned on a rotary microtome, sections were 5µm in thickness, then mounted on glass slides and then deparaffinized in two changes of xylene for 5 minutes each, followed by descending grades of ethanol (absolute, 90%, 80%, and 70%) for two minutes for each. Finally, the prepared sections were stained by routine methods using the Hematoxylin-Eosin (H&E) method.

The stained sections were examined under a light microscope and the different cell and tissue types were carefully studied and photographed. Brain sections from each study group were evaluated for structural changes, and examined by a histologist and a pathologist. Light microscopy (Leica, Germany) was used for the evaluations.[20] All relevant ethical guidelines have been followed and any necessary ethics committee approvals have been obtained.

RESULTS

Vitamin C effect on dementia (histological study)

Group 1: Control 1%T80 (5ml/kg) injected for one week.

Histopathological evaluation was performed on representative H&E sections from the brain mice group injected with 1% T80, revealing normal histological structure for brain parts, cerebral cortex, and hippocampus that shows normal granular and pyramidal layers. Also, normal neurons and neuroglia cells (Figures 1-5).

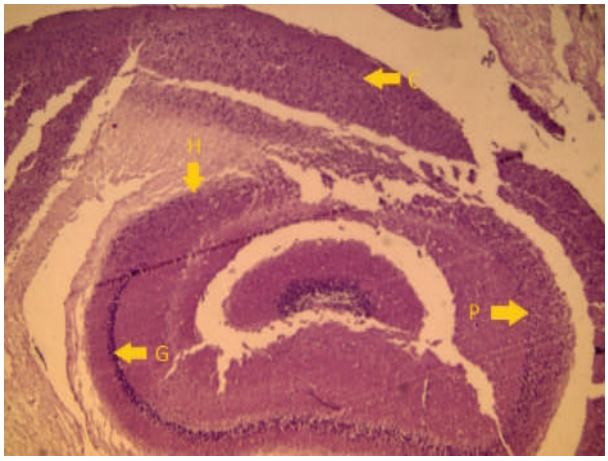

Fig. 1, A photomicrograph of group 1, control mouse brain injected with 1% T80, showing: normal histological structure for brain parts, cerebral cortex (C), and hippocampus (H) which shows normal granular (G) and pyramidal (P) layers. (H&E. 4X).

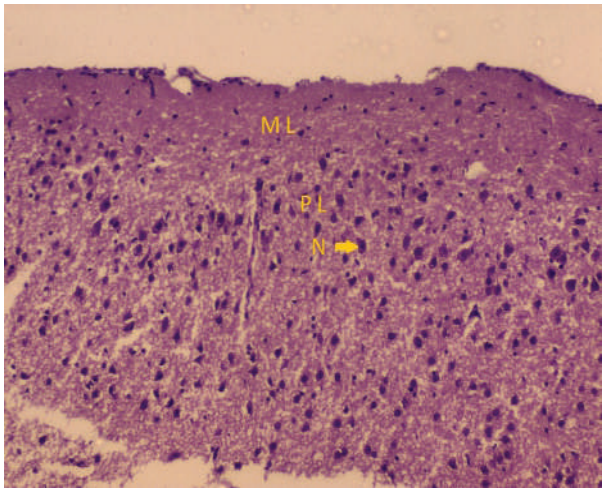

Fig. 2, A photomicrograph of group 1, control mouse brain injected with 1% T80, showing: Normal histological structure for cerebral cortex including molecular layer (M) and pyramidal layer (P), with normal neurons (N). (H&E. 20X).

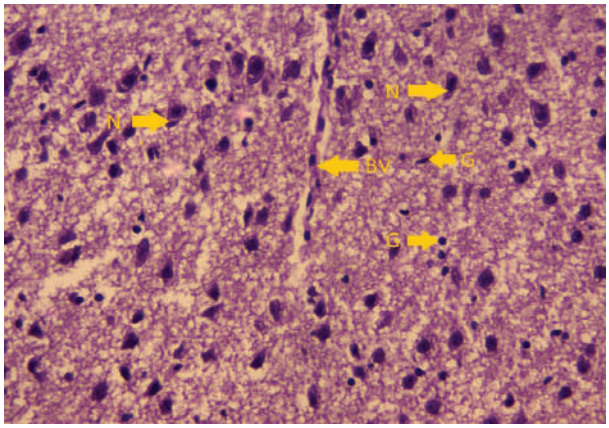

Fig. 3, A photomicrograph of group 1, control mouse brain injected with 1% T80, showing: normal histological structure for cerebral cortex. Normal neurons (N), blood vessels (BV), and glial cells (G). (H&E. 40X).

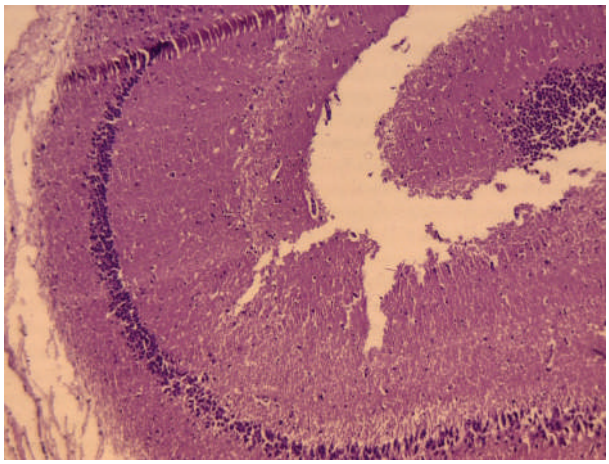

Fig. 4, A photomicrograph of group 1, control mouse brain injected with 1% T80, showing: unremarkable hippocampus (H&E. 10X).

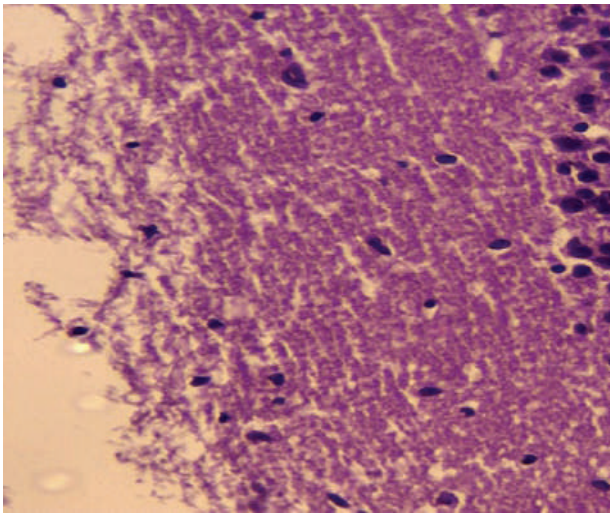

Fig. 5, A photomicrograph of group 1, control mouse brain treated with 1% T80, showing: unremarkable hippocampus (H&E. 40X).

Group 2: Treated with scopolamine HBr

Histopathological observations for the brain of H&E-stained sections for mice treated with scopolamine HBr 6 mg/kg, revealed mild mononuclear cellular (lymphocytic) infiltration and aggregation, mild vascular proliferation, and perivascular edema. Also, the cerebral cortex shows focal gliosis with microglial proliferation and distributed astrocytes. Some shrunken neurons exhibited eosinophilic cytoplasm and shrinkage of basophilic nuclei. The hippocampus was unremarkable (Figures 6-10).

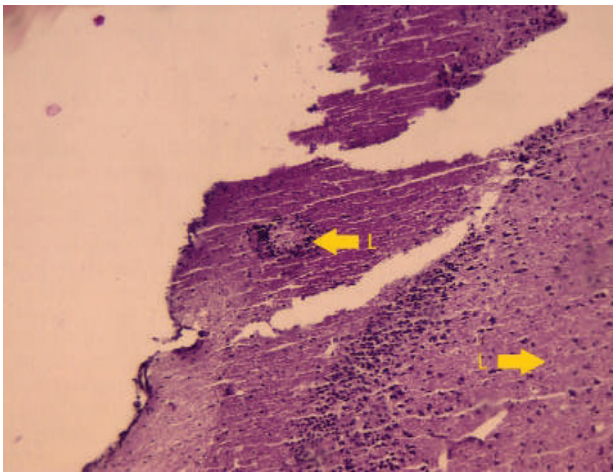

Fig. 6, A photomicrograph of group 2, mouse brain cerebral cortex, injected with scopolamine HBr 6 mg/kg, showing: mild cellular (lymphocytic) (L) infiltration and perivascular (L) aggregation. (H&E. X10).

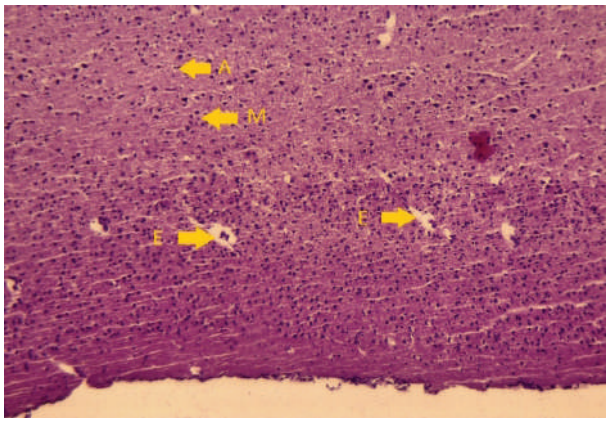

Fig. 7, A photomicrograph of group 2, mouse brain cerebral cortex injected with scopolamine HBr 6 mg/kg, showing: focal gliosis with microglial (M) proliferation and distributed astrocyte. Vascular proliferation with mild perivascular edema (E). (H&E. X10).

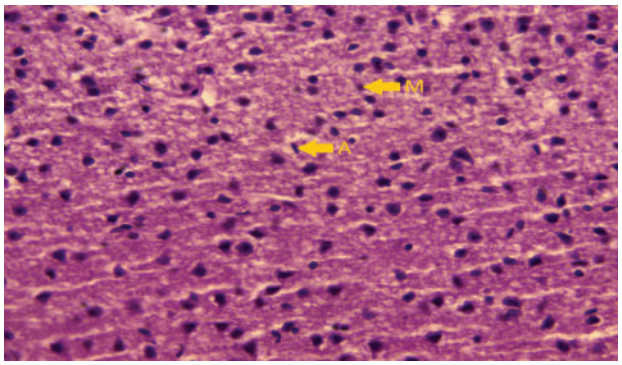

Fig. 8, A photomicrograph of group 2, mouse brain cerebral cortex injected with scopolamine HBr 6 mg/kg, showing: focal gliosis including microglial (M) proliferation and distributed astrocyte (A). (H&E. X40).

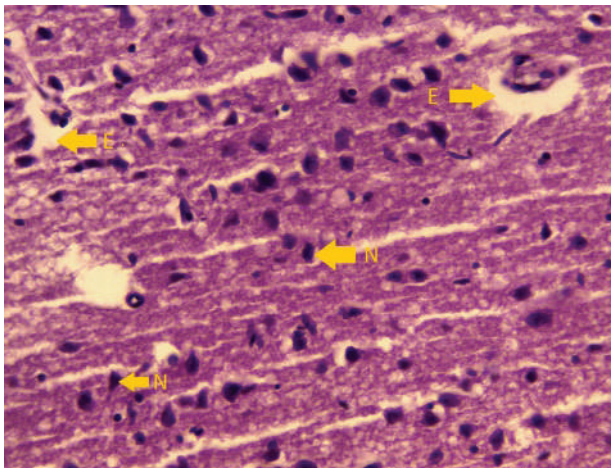

Fig. 9, A photomicrograph of group 2, mouse brain cerebral cortex injected with scopolamine HBr 6 mg/kg, showing: perivascular edema (E). Some shrunken eosinophilic neurons (N) exhibited shrinkage basophilic nuclei. (H&E. X100).

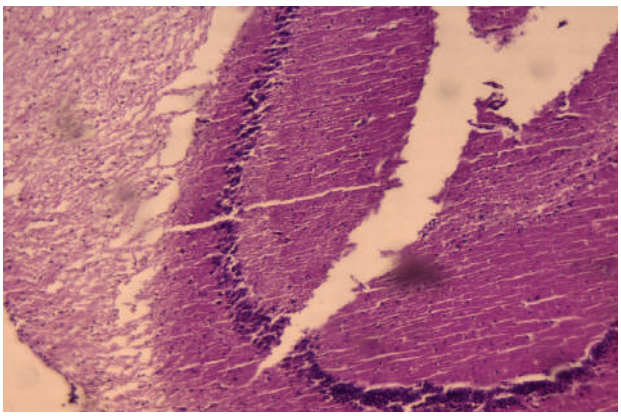

Fig. 10, A photomicrograph of group 2, mouse brain hippocampus injected with scopolamine HBr 6 mg/kg, showing: unremarkable hippocampus. (H&E. X10).

Group 3: Treated with vitamin C

Histopathological observations for brain sections of mice treated with vitamin C revealed mild gliosis with edema and vascular proliferation in the cerebral cortex and hippocampus (Figures 11- 13).

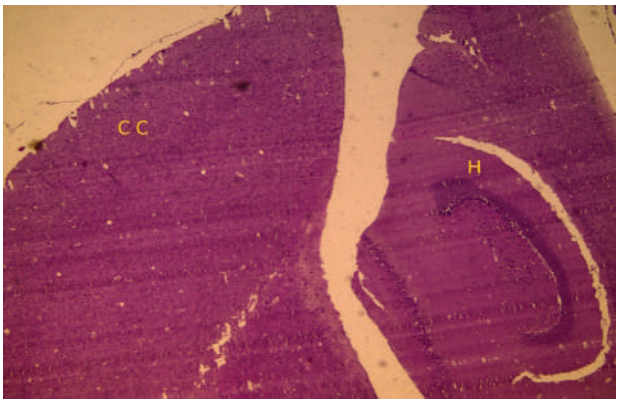

Fig. 11, A photomicrograph of group 3, a mouse brain treated with vitamin C, showed: mild gliosis, and vascular proliferation in the cerebral cortex (CC) and hippocampus (H). (H&E. X10).

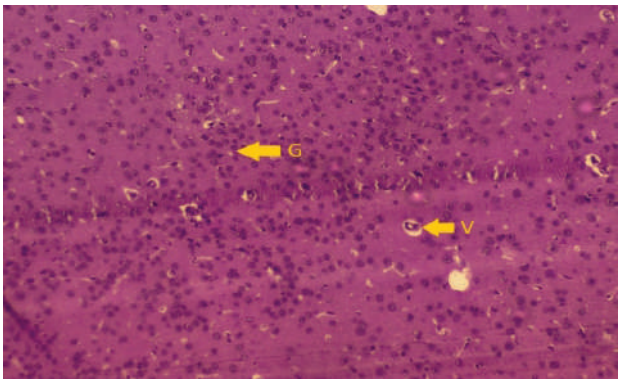

Fig. 12, A photomicrograph of group 3, mouse brain treated with vitamin C, showed: mild gliosis (G) with edema and vascular proliferation (V) in cerebral cortex. (H&E. X10)

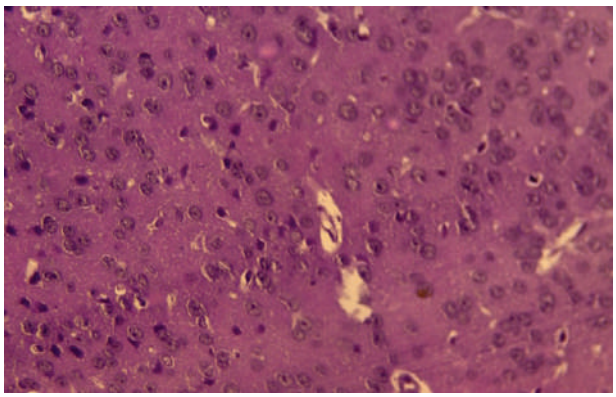

Fig. 13, A photomicrograph of group 3, mouse brain treated with vitamin C, showed: mild gliosis, edema, and vascular proliferation in cerebral cortex. (H&E. X40)

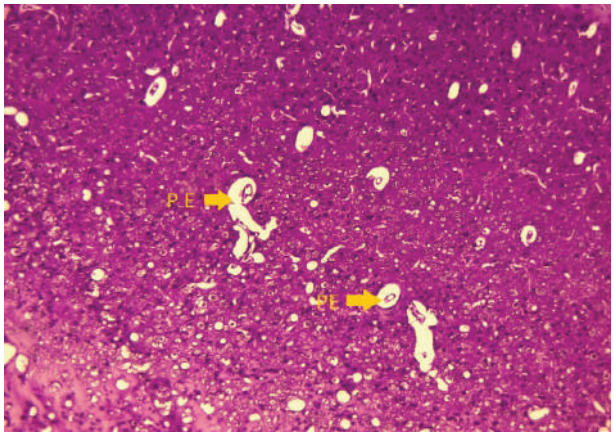

Fig. 14, A photomicrograph of group 4, mouse brain treated with scopolamine HBr and vitamin C, showed: congestion. proliferated blood vessels with perivascular edema (PE) in the cerebral cortex. (H&E. X10).

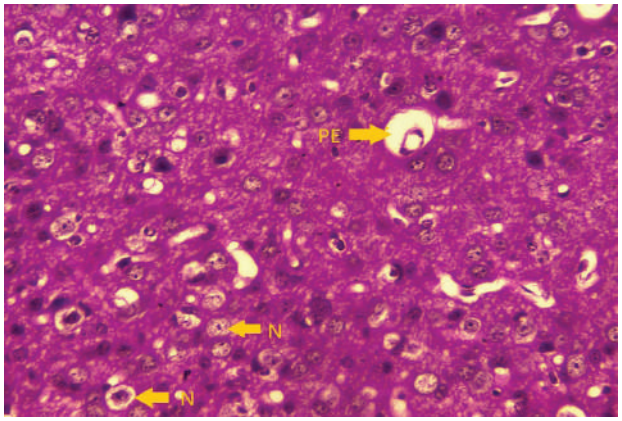

Fig. 15, A photomicrograph of group 4, mouse brain treated with scopolamine HBr and vitamin C, showed: congestion. proliferated blood vessels with perivascular edema (PE) in the cerebral cortex. (H&E. X10).

Group 4: Treated with scopolamine HBr and vitamin C

Histopathological observations for brain sections for mice treated with scopolamine HBr and vitamin C revealed congested proliferated blood vessels with perivascular edema and swollen vacuolated neurons in the cerebral cortex. The hippocampus showed congested proliferated blood vessels with perivascular edema and swollen vacuolated pyramidal neurons (Figures 14-17).

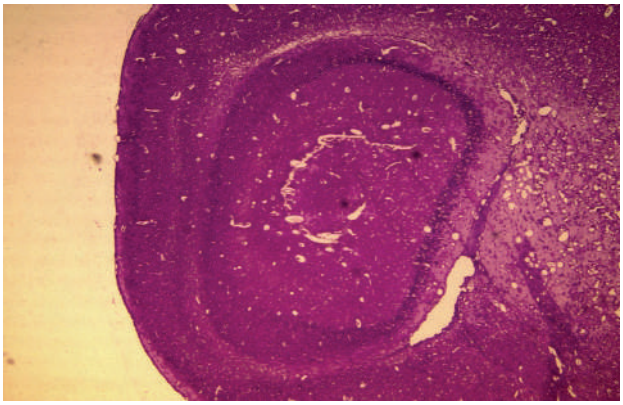

Fig. 16, A photomicrograph of group 4, mouse brain treated with scopolamine HBr and vitamin C, Showed: Congested. proliferated blood vessels with perivascular edema in the hippocampus. (H&E. X4).

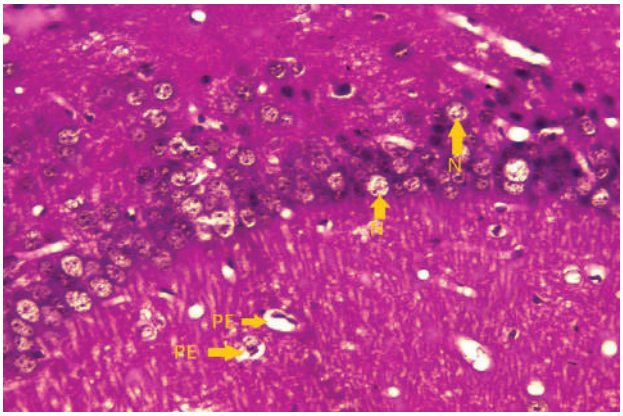

Fig. 17, A photomicrograph of group 4, mouse brain treated with scopolamine HBr and vitamin C, showed: edema, swollen vacuolated pyramidal neurons (N), and perivascular edema (PE) in hippocampus. (H&E.X40).

Group 5: Treated with scopolamine HBr followed by vitamin C

Histopathological observations for brain sections of mice treated with scopolamine HBr followed by vitamin C, revealed that the cerebral cortex showed edema and perivascular proliferation, shrunken, of most neurons with evidence of apoptosis and nuclear fragmentation. The hippocampus showed edema in the pyramidal layer and vascular proliferation. no gliosis and no lymphocytes were observed in both cortex and hippocampus (Figures 18-23).

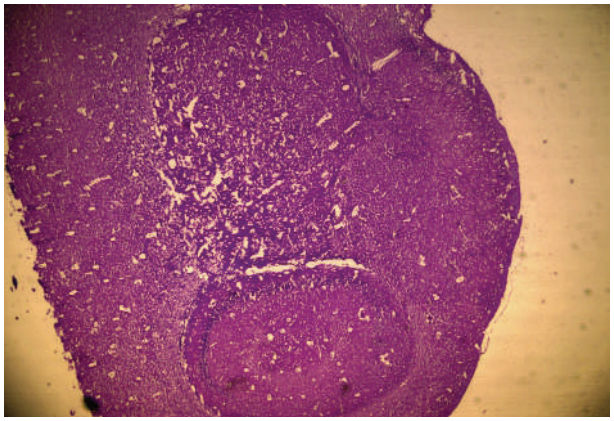

Fig. 18, A photomicrograph of group 5, mouse brain treated with scopolamine HBr followed by vitamin C, showed: vascular proliferation and edema in the cerebral cortex and hippocampus (H&E. X2.5)

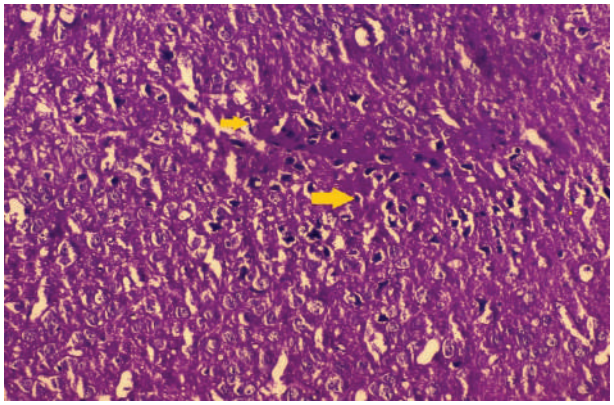

Fig. 19, A photomicrograph of group 5, mouse brain treated with scopolamine HBr followed by vitamin C, showed: shrunken of most neurons and apoptosis. (H&E. X20).

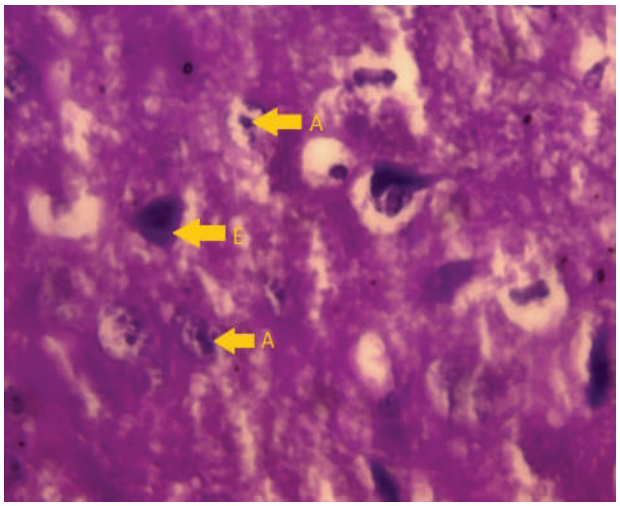

Fig. 20. A photomicrograph of group 5, a mouse brain treated with scopolamine HBr followed by vitamin C, showed: shrunken most neurons, nuclear fragmentation, and apoptosis (A). Some eosinophilic neurons are seen in this section (E). (H&E. X100).

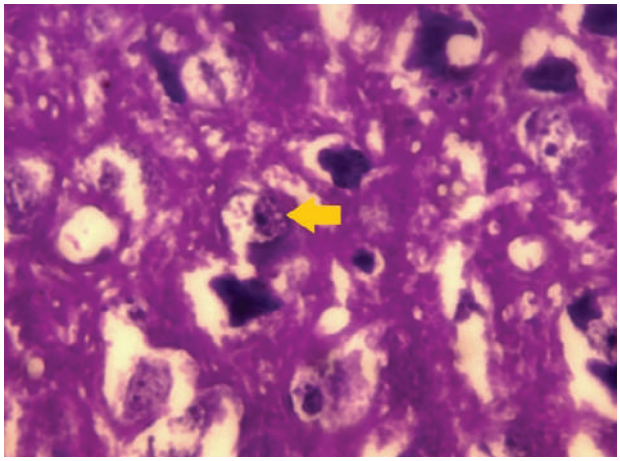

Fig. 21, A photomicrograph of group 5, mouse brain treated with scopolamine HBr followed by vitamin C, showed: shrunken most neurons, nuclear fragmentation, and apoptosis (arrow). Some eosinophilic neurons are seen in this section (E). (H&E. X100).

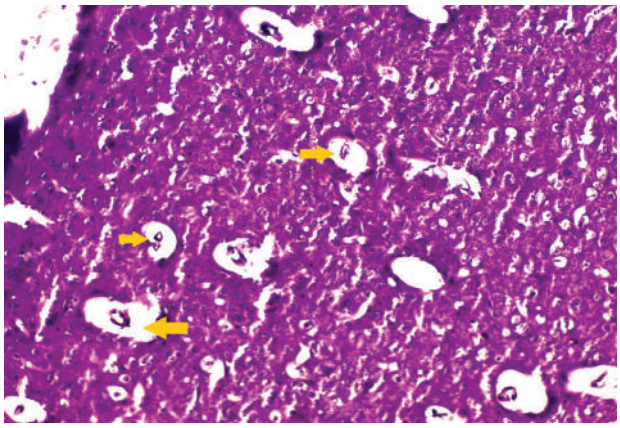

Fig. 22, A photomicrograph of group 5, mouse brain treated with scopolamine HBr followed by vitamin C, showed: perivascular proliferations (arrows) in cerebral cortex. (H&E. X100).

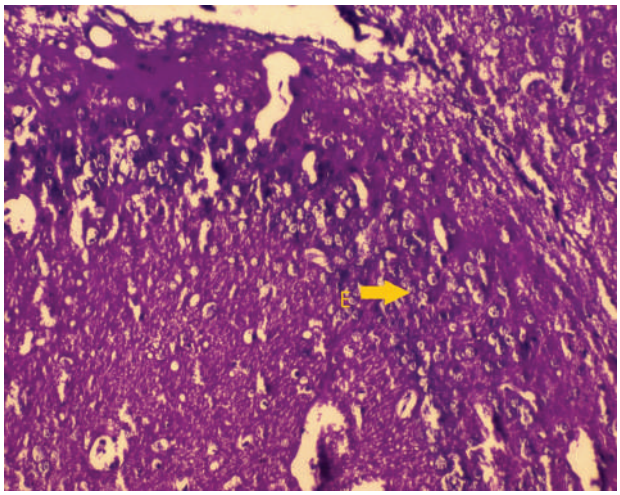

Fig. 23, A photomicrograph of group 5, mouse brain treated with scopolamine HBr followed by vitamin C, showed: edema (E) in the pyramidal layer of hippocampus. (H&E. X100)

Group 6: Treated with scopolamine HBr and donepezil

Histopathological observations for brain sections of mice treated with scopolamine HBr and donepezil, revealed, mild gliosis and lymphocytic infiltration and aggregation in the cerebral cortex. Normal hippocampus (Figures 24 & 25).

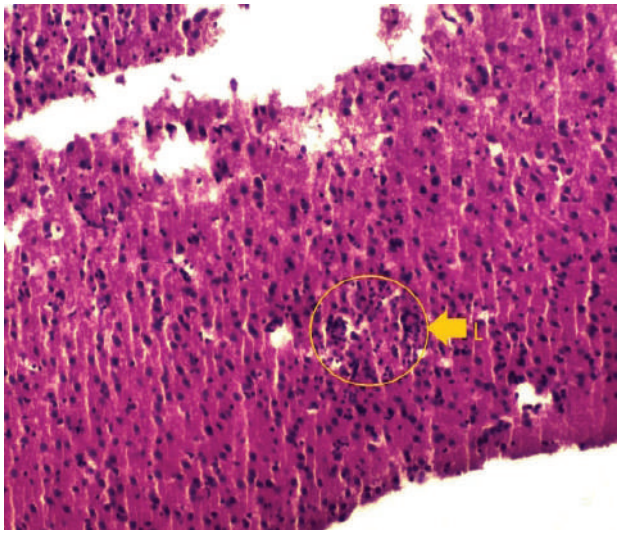

Fig. 24, A photomicrograph of group 7, mouse brain sections treated with scopolamine HBr and donepezil, showed: mild gliosis and lymphocytic infiltration and aggregation (L) in the cerebral cortex (H&E. X20).

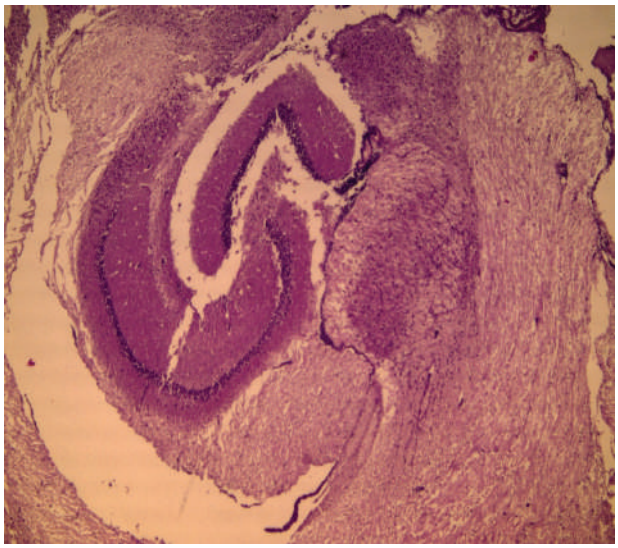

Fig. 25, A photomicrograph of group 7, mouse brain sections treated with scopolamine HBr and donepezil, showed: normal hippocampus (H&E. X20).

Effect of vitamin C on brain volumes of mice after dementia

Brain volumes showed no change after different treatments for one week or two (Table, 1).

Table 1: Effect of vitamin C treatment on brain width and length after dementia induction by scopolamine HBr

Treatments	Width (mm)	Length (mm)
Control (1%T80, 5ml/kg)	13	15
Scopolamine HBr (6 mg/kg)	13	15
Vitamin C (500mg /kg)	13	15
Scopolamine HBr + vitamin C	13	15
Scopolamine HBr followed by vitamin C	13	15
Scopolamine HBr +Donepezil	13	15

DISCUSSION

Dementia was scored using an elevated plus maze (EPM); the transfer latency per mouse will be evaluated.[21,22]

In this work, a scopolamine HBr dose of 6mg/kg was chosen from the pilot study that produces dementia in mice; this dose increases transfer latency by using the elevated plus maze

Dementia was scored using an elevated plus maze (EPM); the transfer latency per mouse would be increased in the case of dementia.[21,22] Scopolamine HBr induced dementia in animals through impaired memory by blocking muscarinic cholinergic receptors in the brain of the animals.[23] Scopolamine HBr competitively inhibits G-protein coupled post-ganglionic muscarinic receptors for acetylcholine, it is a nonselective muscarinic antagonist, it produces peripheral antimuscarinic properties and central sedative, antiemetic, and amnestic effects.[24,25] Scopolamine HBr primarily affects the M1 receptor and has reported H1 receptor activity.[8,26]

Scopolamine HBr is capable of producing deficits in learning acquisition, and consolidation.[27] Scopolamine HBr-treated animal models are used in neurocognitive studies; they induce behavioral and molecular features of Alzheimer's disease and other neurocognitive disorders, such as impaired cognition, and imbalanced cholinergic transmission in the hippocampus and prefrontal cortex.[11,12,28] Scopolamine HBr increased acetylcholinesterase activity, this enzyme is responsible for the synaptic metabolism of acetylcholine, the major neurotransmitter implicated in learning and memory.[29] Therefore, in this work, scopolamine HBr was used to induce dementia through its effect on cholinergic neurons.

Oxidative stress is a key component in neurodegenerative diseases.[30] Oxidative stress markers are associated with dementia.[31] Nitric oxide is a free radical gas; several brain areas including the cerebellum and hippocampus involved in learning and memory are affected by NO.[32] Scopolamine HBr increased the production of nitric oxide (NO) and elevated inducible nitric oxide synthase (iNOS) levels in the cortex and hippocampus of mice, which induce oxidative damage in neurons.[33]

Scopolamine HBr is associated with increased oxidative stress in the brain, especially in the hippocampus and prefrontal cortex areas which are associated with memory and learning function. It also increases pro-inflammatory cytokines, and inflammation has been proposed as the theoretical basis for scopolamine HBr-induced memory impairment.[10,25] In this work scopolamine HBr used to induce dementia may be through an increase of oxidative stress.

In a study by Baek et al, scopolamine HBr increases the expression of amyloid beta (Aβ) and phosphorylated-tau in mice; this led to synaptic dysfunction, neuron loss, and neurodegeneration, causing memory impairment.[34] Scopolamine HBr increases a pro-apoptotic protein (BAX) and decreases the antiapoptotic protein B-cell lymphoma 2 (Bcl2), which induces apoptosis in neurons.[35] Scopolamine HBr increases lipid peroxidation in the brain and reduces glutathione (GSH) levels.[36]

Scopolamine HBr induced a decrease in the expression of Brain-derived neurotrophic factor (BDNF) in rats,[37] and it decreased the level of BDNF in the mice hippocampus. Brain-derived neurotrophic factor has a main role in the function of the brain (especially in learning and memory),[38] where the major receptor for BDNF is tyrosine receptor kinase B (TrkB), the important effects of activation of the BDNF-TrkB pathway is the development of memory and growth of neurons.[39] Therefore, scopolamine HBr may induce dementia through the decrease in the expression and the levels of BDNF in brain mice.

In this study, brain histological analysis revealed serious damaging effects of scopolamine HBr on the structure of the cerebral cortex and hippocampus, which agreed with the last studies of Abd-El-Fattah et al., and Du et al.[36,40] Scopolamine HBr-induced oxidative damage of neurons in hippocampal CA1 and CA3 regions was prevented by antioxidant treatment.[36] In a previous study histopathological examination of the brain hippocampus demonstrated scopolamine HBr-mediated deleterious effects supported by a decrease in pyramidal cells, an increase in vacuolated cytoplasm, and focal gliosis as compared to the control group.[41]

A study demonstrated that scopolamine HBr increases lipid peroxidation in the brain while reducing GSH levels.[36] Another study has demonstrated that vascular dysfunction appears in the first steps of the pathophysiological process leading to dementia. Similarly, a breakdown in the BBB seems to happen particularly early in the hippocampus and correlates to cognitive symptoms.[42] Blood-brain barrier breakdown can interrupt the cerebral blood flow of oxygen and nutrients to the brain, also permitting toxic plasma components, such as blood cells and pathogens entry into the CNS.[43] Animal models have shown that CNS infiltration of neutrophils can induce neuroinflammatory cascades that exacerbate Alzheimer's pathology and cognitive decline.[44]

Oxidative stress is an imbalance between oxidants and antioxidants that causes a rise in oxidant levels. Oxidative stress is well recognized as one of the clinical markers of dementia, it is still unclear whether oxidative stress is a cause of the process that occurs in dementia patients' brains. Reactive oxygen species (ROS) and Aβ are the major mediators of oxidative stress,[45] oxidative stress has been strongly associated with astrocyte swelling.[15] all these changes in brain tissues and edema are related to dementia induced by scopolamine HBr showed in this study confirm with other studies.

In this work, brain histological studies of the vitamin C effect alone showed brain damage with edema. Vitamin C is a hydrophilic molecule, it has two basic barriers to entry into the central nervous system, the first is the blood-brain barrier (BBB), which is very slow.[46] the second is the blood-cerebrospinal fluid barrier (CSF), and then into the brain cells, by the ability to maintain a steep ascorbate concentration gradient from blood to neuronal cells.[47] Its uptake is mainly conditioned by two sodium-dependent transporters, the sodium-dependent Vitamin C transporter type 1 (SVCT1) and type 2 (SVCT2).[46] A study performed on the Alzheimer's mice model, found that oral vitamin C reduced amyloid plaque burden in the cortex and hippocampus by ameliorating BBB disruption via preventing tight junction structural changes.[46]

Vitamin C has pleiotropic effects which a key to circulating antioxidant, anti-inflammatory, immune-supporting effects, and as a cofactor for mono and dioxygenase enzymes.[48] All of these functions are based on electron donation and could decrease cellular and organ injury.[49] Vitamin C is found at 10-fold higher levels in the brain than in plasma, emphasizing the significant role of this antioxidant in the central nervous system.[50] Vitamin C deficiency reduces the reuptake of extracellular glutamate, which in turn may lead to excitotoxic damage in the brain.[47]

Vitamin C is an important neuromodulator for brain health; its deficiency may be associated with an increased risk of dementia.[51] Oxidative stress is an important factor that may be involved in the pathogenesis of dementia. Treatment of these patients with antioxidants may likely slow down the progression of the disease.[50] In the aging brain, deficiency of vitamin C may impair cognitive function through reduced signal transduction, also through amyloid β deposition resulting in the generation of reactive oxygen species.[51]

Prooxidant species in particular oxygen free radicals normally generated during cell metabolism, either as bio-products of several enzymes as a result of the intracellular metabolism of foreign compounds or by ionizing radiation. Cell production of reactive oxygen species (ROS) can exceed the endogenous antioxidant defenses, leading to oxidative stress and consequently to the oxidation of cellular macromolecules. Oxidative stress is a feature common to aging and several degenerative diseases.[52] A study demonstrates that the pro-oxidant action of vitamin C at lower concentrations may be due to their stimulation of oxidative effects, and it causes strand breakage in DNA in the presence of oxygen and can induce cell death.[53]

The pathogenesis of brain edema is classified as vasogenic or cytotoxic edema. Vasogenic edema is an extracellular accumulation of fluid resulting from disruption of the blood-brain barrier (BBB), and extravasations of serum

proteins, while cytotoxic edema is characterized by cell swelling caused by intracellular accumulation of fluid.[54] In clinical studies, vitamin C as supplements in doses of 500 mg or more per day acts as an antioxidant,[55] while vitamin C at a dose less than 500mg/kg (200mg/kg) may act as a pro-oxidant.[55,56] Research suggested that three factors are responsible for the pro- or antioxidant behavior of vitamin C in biological systems; the first is the redox potential of the cellular environment (oxidosis/redosis), the second is the presence or absence of transition metals, and the third is the local concentration of ascorbate. In vivo vitamin C may act more as a pro-oxidant than an antioxidant, possibly necessitating increased activation of the defense system to maintain a stable status.[57]

In the present study, vitamin C as a protective effect has not any improvement in brain histological studies compared to scopolamine HBr, which showed swollen neurons. Dementia and vitamin C at low concentrations may cause damage specifically followed by an increase in the sodium permeability, which exceeds the capacity of the pump to extrude the sodium. Accumulation of sodium in the cell leads to an increase in water content in the cell leading to its swelling.[58] Ascorbate release from astrocytes, glial cells, and neurons depends on the increase of reuptake of glutamate on one or more of its specific transporters. This process is initiated either by the activation of glutamatergic pathways or by direct infusion of glutamate.[47,59]

The mechanism of glutamate-induced ascorbate release also brings sodium into the cell, which causes swelling of astrocytes.[60] Ascorbate at high concentration has been shown to protect neurons from excitotoxicity induced by activation of the NMDA receptor, and it prevented glutamate-induced cell damage and death. This could be due to redox modulation of the receptor itself by ascorbate, or to direct scavenging of ROS generated by NMDA receptor activation.[47]

Scopolamine HBr induced memory impairment, due to long-lasting oxidative stress which may damage the hippocampus tissue.[27] Vitamin C will be more consumed as an antioxidant,[61] leading to an excessive decrease in the levels of vitamin C, as a result, vitamin C, with low levels, will produce damage instead of protection.

High concentrations of ascorbic acid and an increase in cerebral blood flow led to reductions in neurological deficits.[62] A high dose of vitamin C can prevent oxidative stress, it can reduce blood-brain barrier permeability, brain edema, and neuron apoptosis.[63] In clinical trials, administration of low-dose vitamin C did not cause any improvement in dementia, and edema increased. However, high-dose vitamin C decreases the progression of perilesional edema in the brain caused by oxidative stress.[62] Brain edema may lead to an imbalance in energy demand and influence the postsynaptic effects of glutamate (excitotoxicity).[64] Excitotoxicity induced by activation of the NMDA receptor and glutamate-induced cell damage and death in nerve cells. This could be due to oxidative stress or ROS generated by NMDA receptor activation.[47]

A study demonstrated high dose of vitamin C act as neuroprotective in the striatum but not in the hippocampus of rats, this action is mediated neither by blockade of the NMDA receptor nor modulation of its redox status, but it may be due to vitamin C delayed or diminished dopamine release.[65] In another study by kook et al., chronic administration with higher dose supplementation of vitamin C decreased the plaque burden in the cortex (by 57.9%) and hippocampus (40.29%) of mice with dementia.[66] Improvement effect of dementia by vitamin C administration depending on different doses, and mechanisms in different brain areas.

Oxidative stress has been implicated as a potential contributor to the pathogenesis of dementia, free radicals can cause damage to cellular lipids, proteins, and nucleic acids, leading to subsequent cell death by modes of necrosis or apoptosis. Oxidative stress can block apoptosis while vitamin C can protect against this oxidative stress and allow progression of the apoptotic process, while higher oxidant concentrations can cause necrotic cell death, and then vitamin C may not mediate protection of apoptosis, so necrosis promotes inflammation and is to be avoided.[67] In clinical study showed that a high dose of vitamin C administration has been associated with reduced oxidative stress and improved neurological function.[49]

The antioxidant activities of vitamin C at a higher concentration were mainly due to the scavenging of hydrogen peroxide in the system. The pro-oxidant mechanism for vitamin C at lower concentrations is due to the strong electron-donating effects (reducing power) and weak metal chelating ability, as well as their stimulation of oxidative effect.[53] Vitamin C has a switchover role from being an antioxidant in physiologic conditions to a pro-oxidant under pathologic conditions.[68]

Vitamin C contributes to protecting neutrophils from oxidative stress during the early stages of an immune response when neutrophils activate phagocytosis and produce reactive oxygen species (ROS) to destroy antigens. Once the phagocytic capacity is exhausted and neutrophils start to die, vitamin C seems to regulate the process in favor of apoptosis, through the activation of a caspase-dependent cascade, inhibiting the transition to necrosis, and resulting in a more efficient decrease of inflammation.[69]

Necrosis promotes inflammation and should be avoided. Vitamin C may act as an apoptosis regulator and this is dependent on the level of oxidative stress exposure. Vitamin C-mediated protection of apoptosis was marked at a low level of exposure to oxidative stress but was ineffective at a higher level of exposure to oxidative stress. This could indicate that the antioxidant or repair capacity of vitamin C can be overwhelmed by higher oxidant concentrations, or at these levels cause necrotic cell death.[67] In this work, histological studies showed worsened damage in vitamin C treatment groups compared to the scopolamine HBr treated group; where appears apoptosis (cell fragmentation) and edema.

Among brain areas, the hippocampus, occipital, and frontal cortex have the highest levels of vitamin C, while vitamin C deficiency leads to abnormalities in brain development, including a 10-15% decrease in hippocampal volume and

learning/memory impairments.[59] In the developing brain, neuronal density and maturation are compromised by vitamin C deficiency, giving rise to decreased brain volume.[51] In clinical studies, the cognitive decline in dementia is found to be associated with brain volume reductions,[70,71] even in those in the early stage of dementia.[72]

In this study, dementia was observed after only scopolamine HBr administration for one week without any change in brain volumes. As mentioned earlier, dementia is a chronic syndrome,[73] in dementia, the same amount of brain damage or pathology may have different effects on different people, even when brain size is held constant.[74] Vitamin C treated groups showed no effect on brain volumes compared to the control treated group. Therefore, brain volume changes were not observed in this study.

CONCLUSION

The dementia model induced by scopolamine HBr, showed mild mononuclear cellular (lymphocytic) infiltration and aggregation, mild vascular proliferation, and perivascular edema. In the cerebral cortex, focal gliosis with microglial proliferation and distributed astrocytes was observed. Some shrunken neurons exhibited eosinophilic cytoplasm and shrinkage of basophilic nuclei. The hippocampus was unremarkable.

Administration of vitamin C alone, in the dose used, acts as a pro-oxidant, it produces damage in the cerebral cortex and hippocampus; the damage is observed as a mild gliosis with edema and vascular proliferation.

The combined administration of vitamin C and scopolamine HBr produced more damage, the brain histology showed congested proliferated blood vessels with perivascular edema and swollen vacuolated neurons in the cerebral cortex. The hippocampus showed congested proliferated blood vessels with perivascular edema and swollen vacuolated pyramidal neurons.

The brain damage observed when vitamin C was administered after scopolamine HBr was more severe. It showed edema and perivascular proliferation, shrunken of most neurons with evidence of apoptosis and nuclear fragmentation in the cerebral cortex; while in the hippocampus, the damage showed edema in the pyramidal layer and vascular proliferation.

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