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Evaluation of Neuroprotective Role of Coenzyme Q 10 in Attenuating Changes of Hippocampus Induced by Diet Coke Consumption in Adult Male Albino Rats

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Abstract

Background: **Diet Coke contains a non-nutritive sweetener; aspartame may lead to memory loss and changes in behavior. Coenzyme Q 10 is a powerful endogenous antioxidant within the inner membrane of mitochondria. It has a neuroprotective effect against lipid peroxidation and impairment of memory in aged animals.** ***Aim:*** **To evaluate Coenzyme Q 10's ameliorative effects on the histopathological changes caused by Diet Coke consumption in adult male albino rats, using histological and immunohistochemical techniques.** ***Subjects and Methods:*** **40 adult male albino rats were equally randomized into 4 groups and received all treatments for 21 days. Group I, control. Group II, Coenzyme Q 10; a daily oral dose of 200 mg/kg was administered. Group III was given orally twice a day, 2.5 ml of diet Coke. Group IV, Coenzyme Q 10 with Diet Coke was given concomitantly. All animals were sacrificed after 21 days. Sections stained with Hematoxylin and Eosin stain, Mallory's phosphotungestic acid hematoxylin (PTAH), and Cresyl fast violet stains. Glial fibrillary acidic protein (GFAP) and caspase 3 immunostaining were also used.** ***Results:*** **Coenzyme Q 10 reversed almost all the histopathological changes induced by Diet Coke and decreased caspase 3 and GFAP immunoreaction, in the hippocampus CA3 region.** ***Conclusion:*** **Coenzyme Q 10 has an ameliorative role in attenuating histopathological changes in the hippocampus induced by Diet Coke consumption in adult male albino rats.**

Keywords: *Pyramidal cells, GFAP, Caspase 3, Aspartame.*

Introduction

It was reported that one of the main limbic system nuclei; is the hippocampus, which is medially positioned in the temporal lobe. Although it is traditionally known as the center of memory in the brain, it is suggested to be important in spatial cognition and emotional behavior. It might be affected by multiple psychiatric and neurological disorders(1). It was reported that children, teens, and adults are all consuming fast food. Diet Coke and other carbonated beverages are frequently consumed with fast food(2). Carbonated beverages were reported to be the second most-

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used drink worldwide. Besides their harmful effects on teeth, bone, and vessels of the heart, confirmed the dangerous consequence of soft drinks on gene expression and liver function in rats[3]. Based on company stipulations, Coca-Cola is a carbonated solution of water consisting of caffeine 11.5 mg, carbohydrates 10.6 g, sodium 7 mg, caramel, phosphoric acid, citric acid, lime juice, extract of vanilla and fluid coca extract, and natural flavorings, in 100 ml. It was mentioned that regular Cola is different from light one by the presence of aspartame; a non-nutritive sweetener about (24 mg/100 ml) instead of carbohydrates[4]. Aspartame was reported to cause behavioral changes and loss of memory[5]. Additionally, Abd El-Wahed *et al.* 2019[2] found that Diet Coke resulted in significant degenerative changes in the rat cerebellar cortex. Thus, finding some protective agents against changes induced by Diet Coke consumption was important. Coenzyme Q 10 (or ubiquinone) was suggested to be a strong endogenous antioxidant located in the internal mitochondrial membrane. It was observed that Q10's ability to scavenge free radicals, thus protecting mitochondria and lipid membranes. Additionally, it is crucial to maintain respiratory chain complexes within mitochondria. Moreover, it was also reported to have extramitochondrial actions, including autophagy, ferroptosis, and regulation of the cell membrane's physiological and chemical characteristics[6,7]. Aaseth, Alexander, and Alehagen (2021) found that patients with neurodegenerative disorders have lowered levels of Coenzyme Q 10 in their plasma[8]. Additionally, Hosseini *et al.* 2022[9] found that oral administration of Q10 reversed aging-related memory impairment. They attributed their results to the ability of Q10 to partially reverse the reduced ATP production, mitophagy-proteins such as (PINK, Parkin, and P62 levels and LC3II/I ratio), apoptosis, lipid peroxidation, high levels of reactive oxygen species in aged animals hippocampus. Some research was directed to study the effect of soft drink consumption on the hippocampus. Most of them pursued different methodologies. However, no available studies demonstrated the role of coenzyme Q 10 in attenuating these effects. Hence, the current work was designed to assess the role of coenzyme Q 10 in attenuating changes in the hippocampus induced by Diet Coke consumption in adult male albino rats using histological and immunohistochemical techniques.

Material and Methods

The Suez Canal University Faculty of Medicine (FOM, SCU), where this experimental comparative study was carried out in the Histology and Cell Biology Department. Animal care was carried out under its institutional policies (Code number: 5289).

Experimental animals

Forty adult male albino rats weighing between 150 and 180 g and of the same age (3 months) were employed in the present study. Rats were purchased from Animal House, Center of Excellence (FOM, SCU). They were kept for one week before the beginning of the experimental work for acclimatization. At room temperature and with adequate ventilation, rats were kept in stainless steel cages, supplied with a standard balanced diet and unlimited access to water.

Drug preparation

Diet Coke was purchased from the market. It was kept at room temperature, after degassing by intense shaking[10]. *Coenzyme Q 10* was obtained from Puritan's Pride, in the form of a jar containing 60 rapid-release soft gels, each containing 100 mg of Coenzyme Q 10. (N.B. All were prepared imme-

diately before administration). Thermo Scientific Company, Neomarks, Fremont, USA, provided *the primary rabbit anti-Glial Fibrillary Acid Protein (anti-GFAP) antibody and the rabbit polyclonal cleaved caspase-3 antibody, RB-1197-P0(Ab4).* The supplier was Sigma-Aldarich Chemicals.

Experimental design

Rats were randomly subdivided by using a random number table into 4 groups (10 animals each). All treatments were given for 21 days. *Group I* (the control group) consisted of 10 rats which were separated into two equal subgroups, each containing 5 rats. Group Ia: negative control group, in which the animals were not given any therapy. Group Ib: The positive control group was administered olive oil daily by gavage at a dose of 0.5 ml[11]. *Group II* (Coenzyme Q 10 group): Animals of this group were given daily coenzyme Q 10 dissolved in olive oil, by oral gavage, in a dose of 200 mg/kg[12]. *Group III* (Diet Coke group): 2.5 ml of diet Coke two times daily, was given to each rat of such a group, 6 hours apart, by oral gavage[2]. *Group IV* (Coenzyme Q 10 + Diet Coke group): Rats of this group received coenzyme Q 10 concomitant with diet Coke with the same previous doses.

Tissue sample collection

At the end of the experimental work, every group fasted for the entire night. After that, the rats were decapitated and given thiopental sodium intraperitoneally at a dose of 30 mg/kg to induce general anesthesia before they were killed[13]. Then the skulls were carefully opened. The brain was extracted with great care, quickly dissected, and then processed for both histological and immunohistochemical techniques[14].

Light microscopic study

Serial sagittal sections of the brain were cut and fixed in formalin (10%) for 48 hours. They were subsequently dried in increasing amounts of alcohol, cleared in xylene, and ultimately embedded in paraffin for preparation of 5µm thick sections to be stained with the following stains[15]: *1. Hematoxylin and eosin (H&E):* to display the hippocampal general architecture. *2. Mallory's phosphotungestic acid hematoxylin (PTAH) staining:* for dendrite arborizations. *3. Cresyl fast violet:* for demonstration of Nissl's granules.

Immunohistochemical study

The immunohistochemical detection of glial fibrillary acidic protein (GFAP) and caspase-3 was performed using primary rabbit anti-rat caspase-3 and main rabbit anti-GFAP antibodies, respectively. The method of avidin-biotin complex was used. The large pyramidal cells' cytoplasm was shown to be brown in the presence of a positive caspase 3 response, and the astrocytes' bodies and processes were brown in the presence of a positive glial fibrillary acidic protein reaction. The protocols for the negative controls were the same, except for using phosphate-buffered saline in place of the main antibodies[16]. Using 5 high power fields (x 400 for H&E-stained sections and x 630 for sections stained with GFAP & caspase 3 immunostaining), 10 serial sections from each animal in all groups were examined for qualitative assessment. 10 MP (megapixels) (3656 x 2740 pixels) was the resolution of each image, which was taken by the camera of a calibrated digital standard microscope; Tucson ISH1000 digital microscope camera and an Olympus CX21 microscope. Images were captured and enhanced using the "IS Capture" program.

Morphometric study

Software Image J was applied for quantitative measurements of the area percent of Nissl's granules in cresyl violet-stained sections, using a magnification of 400 in different groups. Additionally, using a magnification of 630, the area percent for GFAP & caspase 3 immunoreactions was measured in the hippocampus tissue in different studied groups.

Statistical Analysis

Microsoft Excel from the Microsoft Office 365 Software Package (from Microsoft Corporation, USA) was used for data entry. Data entry was made for the mean and standard deviation (SD). Data were then subjected to one-way analysis of variance (ANOVA) and the post hoc Tukey test to detect differences in histopathological abnormalities between groups. To evaluate statistical significance, a P-value of under 0.05 was applied.

Results

In the CA3 region, sections of groups Ia (negative control group) and II (Coenzyme Q 10 group) stained with hematoxylin and eosin revealed a layer of large pyramidal cells with vesicular nuclei, an obvious nucleolus, and dendrites (Figures 1 A & B). Group Ib (Positive control group) was nearly like the negative one. In group III (Diet Coke group), there were many degenerated pyramidal cells (Figure 1C). In group IV (Coenzyme Q 10+Diet Coke group), the restoration of normal pyramidal cells with open-face nuclei in the CA3 region was shown (Figure 1D). Purple Nissl's granules in the perikarya of the pyramidal cells appeared normally in the CA3 region of the hippocampus when stained with Cresyl fast violet from the Coenzyme Q 10 group and negative control group (Figures 2 A & B). The Positive control group was nearly similar to the negative one. The perikarya of pyramidal cells of the Diet Coke group showed a significant decrease in purple Nissl's granules in comparison to the control group (Figure 2C & Figure 3). Coenzyme Q10 + Diet Coke group showed increased purple Nissl's granules in the perikarya of pyramidal cells which showed statistical difference in comparison to the Diet Coke group (Figure 2D & Figure 3). Regarding the PTAH stain of the hippocampus CA3 region, the negative control & Coenzyme Q10 groups showed blue-stained dendrite arborizations of the pyramidal cells (Figures 4 A & B). The Positive control group was nearly similar to the negative one. The Diet Coke group showed decreased dendrite arborizations of the pyramidal cells (Figure 4C). In the Coenzyme + Diet Coke group, restoration of dendrite arborizations of the pyramidal appeared (Figure 4 D). In the hippocampus CA3 region stained with GFAP immunostaining, there was weak positive brownish immunoreaction of the astrocytes in between pyramidal cells in both negative control and Coenzyme Q10 groups (Figures 5 A & B). Immunoreaction of the positive control group was almost as that of the negative one. Diet Coke group, showed strong positive cytoplasmic immunoreaction in the astrocytes evidenced by an increase in the number, processes, and size of positive brownish star-shaped astrocytes between pyramidal cells (Figure 5C). This was statistically significant compared to the control group (Figure 6). Coenzyme Q10 + Diet Coke group showed a decrease in the number, processes, and size of positive brownish immunoreactive star-shaped astrocytes (Figure 5D), in comparison to that of the Diet Coke group. This showed a statistical difference in comparison to that of the Diet Coke group (Figure 6).

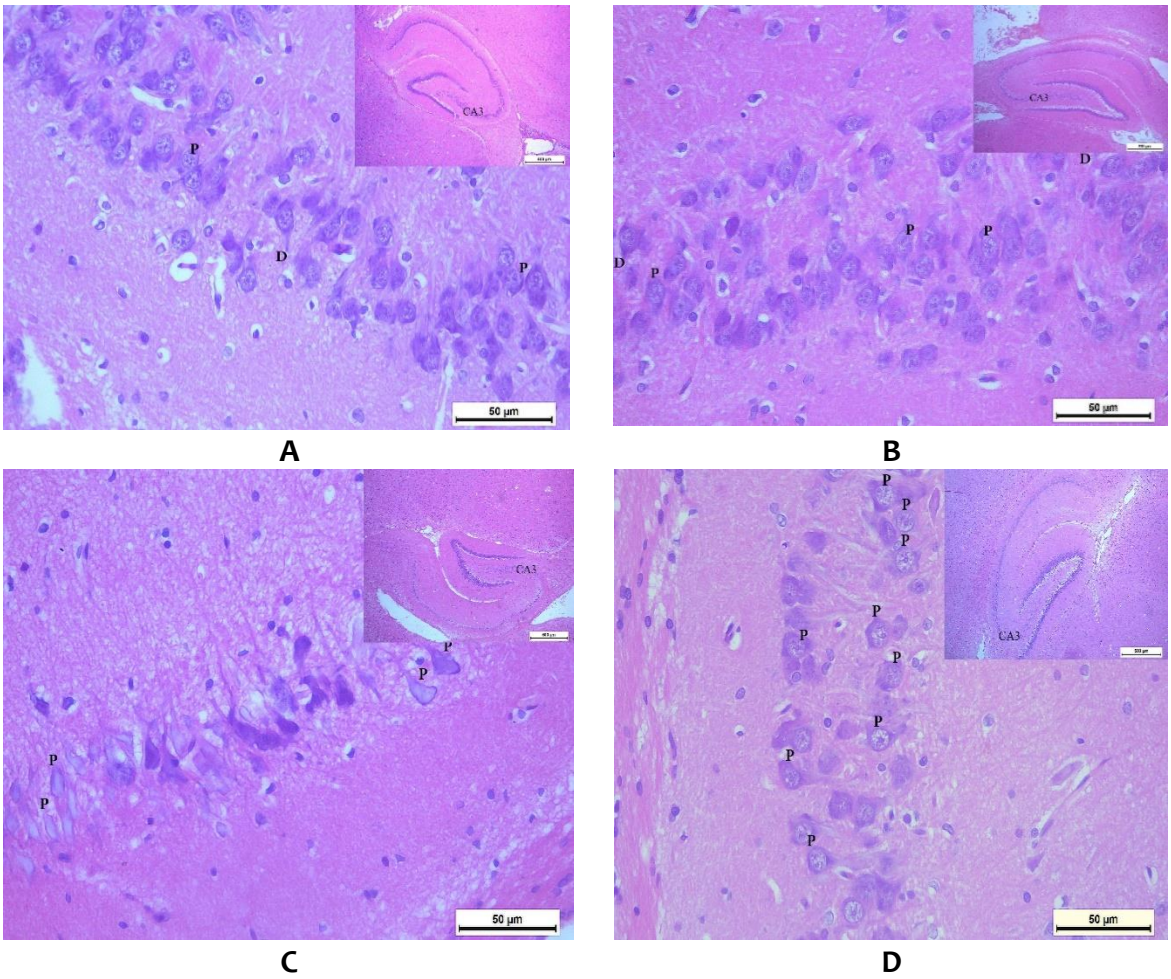

Figure 1: Photomicrographs of the hippocampus CA3 region stained with H&E X 400 from different groups with insets x100 show CA3 in this low power. ***(A)*** *[Negative Control group] shows the CA3 region with a layer of large pyramidal cells (P) which have vesicular nuclei and prominent nucleoli. Dendrites (D) are also shown.* ***(B)*** *[Coenzyme Q10 group] is almost as the control group.* ***(C)*** *[Diet Coke group] shows many degenerated pyramidal cells (P) with no obvious nuclei.* ***(D)*** *[Coenzyme Q10 + Diet Coke group] shows restoration of the hippocampus CA3 layer with many normal pyramidal cells (P) with vesicular nuclei and prominent nucleoli.*

In the hippocampus CA3 region stained with caspase 3 immunostaining in the negative control group, there was negative brown cytoplasmic immunoreaction of the large pyramidal cells (Figure 7A). Immunoreaction of the positive control group was almost as that of the negative control group. The immunoreaction of pyramidal cells in the Coenzyme Q 10 group was more or less similar to that of the control group (Figure 7B). In the Diet Coke group, there was an increased brown cytoplasmic reaction of the large pyramidal cells (Figure 7C). This was statistically significant in comparison to control rats (Figure 8). Coenzyme Q10 + Diet Coke group showed decreased brown cytoplasmic reaction of the large pyramidal cells (Figure 7D), This was confirmed statistically and showed a statistical difference compared to the Diet Coke group (Figure 8).

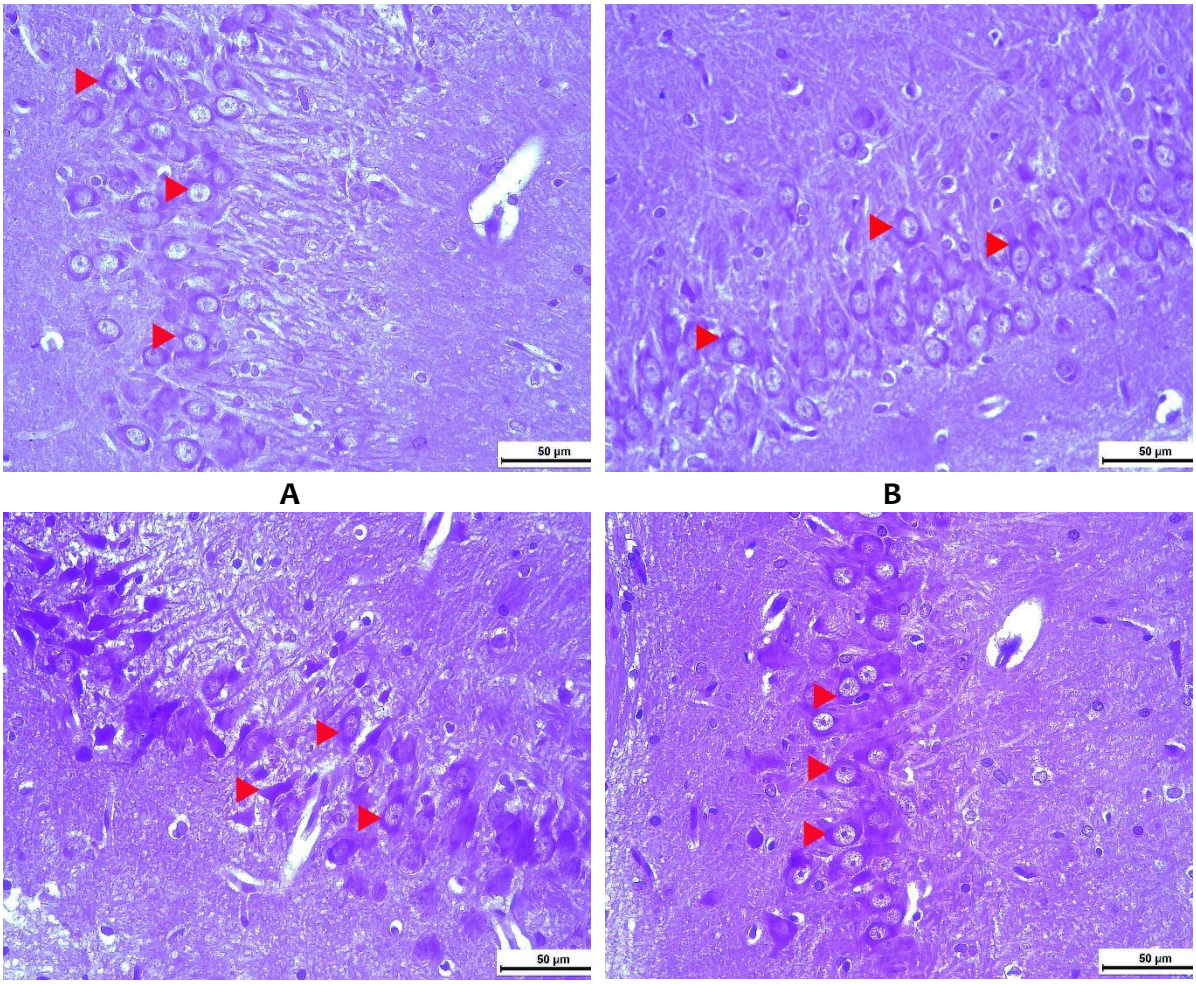

Figure 2: Photomicrographs of the hippocampus CA3 region stained with Cresyl fast violet X 400 from different groups. ***(A)*** *[Negative control group] shows purple Nissl's granules in the perikarya of the pyramidal cells (arrowheads).* ***(B)*** *[Coenzyme Q10] is almost as the control group (arrowheads).* ***(C)*** *[Diet Coke group] shows decreased purple Nissl's granules in the perikarya of pyramidal cells (arrowheads).* ***(D)*** *[Coenzyme Q10 + Diet Coke] shows increased purple Nissl's granules in the perikarya of pyramidal cells (arrowheads).*

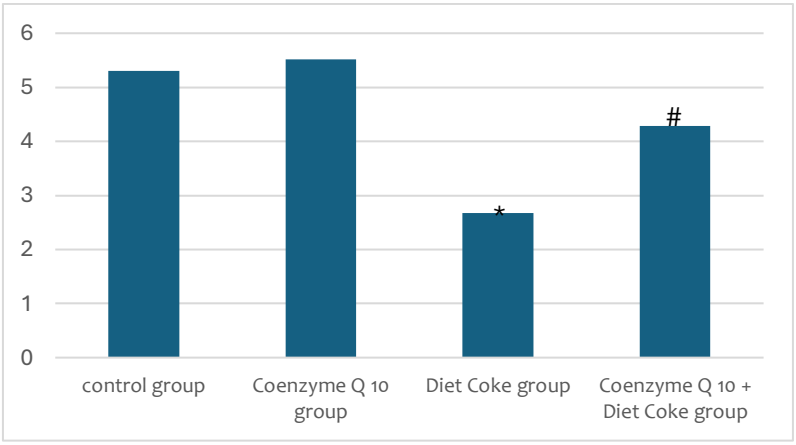

Figure 3: The mean area percent of Nissl's granules in the different groups.
* Statistically significant compared to the control group (P<0.01,
# Statistically significant compared to the Diet Coke group (P<0.01)

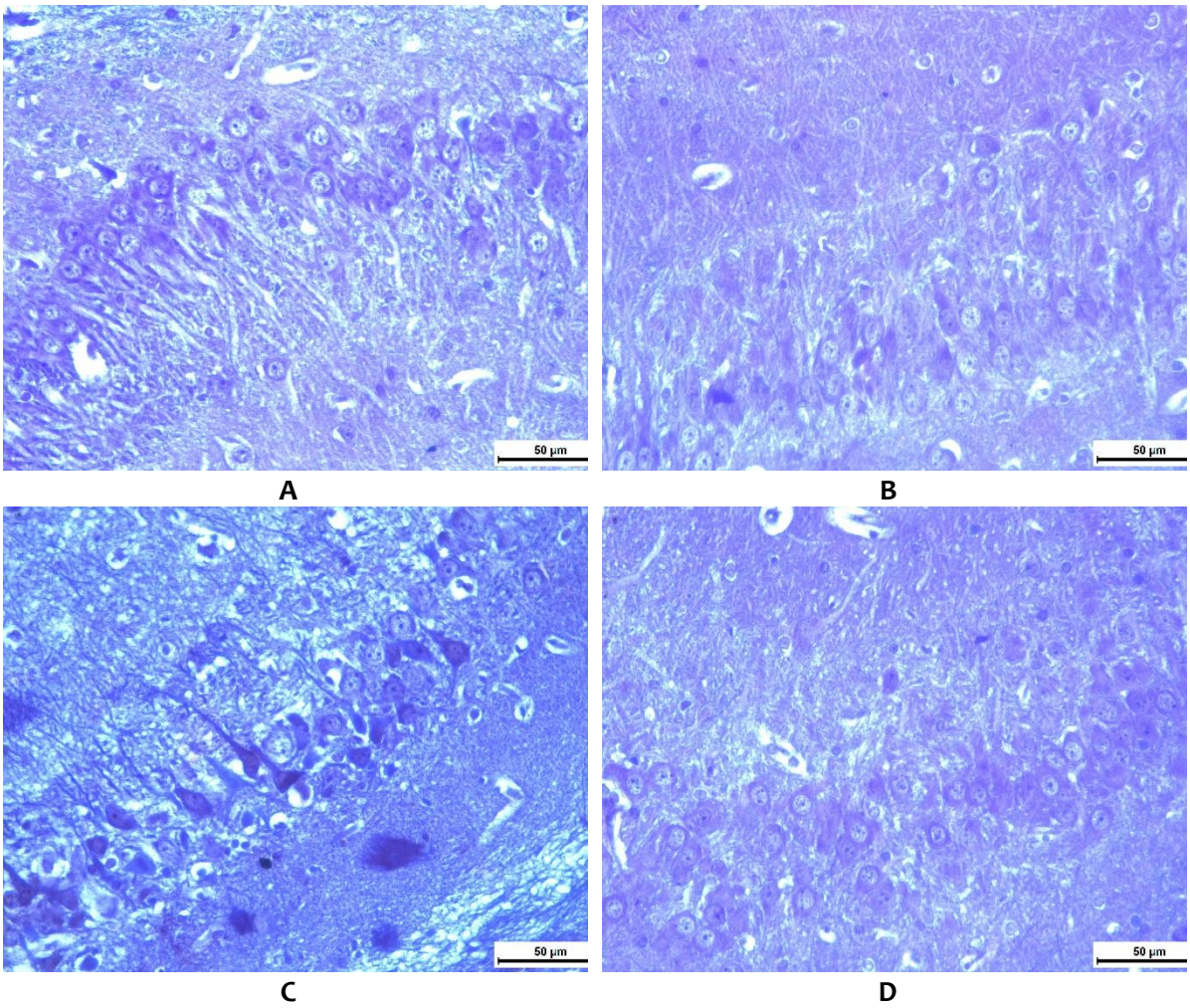

Figure 4: Photomicrographs of the hippocampus CA3 region stained with PTAH X 400 from different groups. ***(A)*** *[Negative control group] shows blue-colored dendrite arborizations of pyramidal cells.* ***(B)*** *[Coenzyme Q10 group] shows pyramidal cells' dendrite arborization similar to the negative control.* ***(C)*** *[Diet Coke group] shows decreased arborization of pyramidal cell dendrites.* ***(D)*** *[Coenzyme + Diet Coke group] shows dendrite arborizations of pyramidal cells, nearly similar to the control group.*

Discussion

Diet Coke is one of the most widely used beverages worldwide. Aspartame (ASP), the main substance it contains, has a sweetness level of around 200 times that of sucrose. In the intestine, ASP is hydrolyzed to its primary ingredients, aspartic acid, methanol, and phenylalanine. All these components are harmful and can impact several body systems, including the central nervous system. Phenylalanine, which makes up around 50% of aspartame, can pass across the blood-brain barrier (BBB), and function as a precursor of catecholamines in the brain causing phenylketonuria. Aspartic acid, which is thought to be an excitotoxin and may contribute to free radical damage in the brain, makes up 40% of aspartame. 10% of the aspartame byproducts are methanol, a poisonous substance that the liver converts to neurotoxic and cancer-causing formaldehyde[17]. Numerous investigations have revealed a link between long-term ASP use and a variety of neurobehavioral and neuropsychiatric problems.

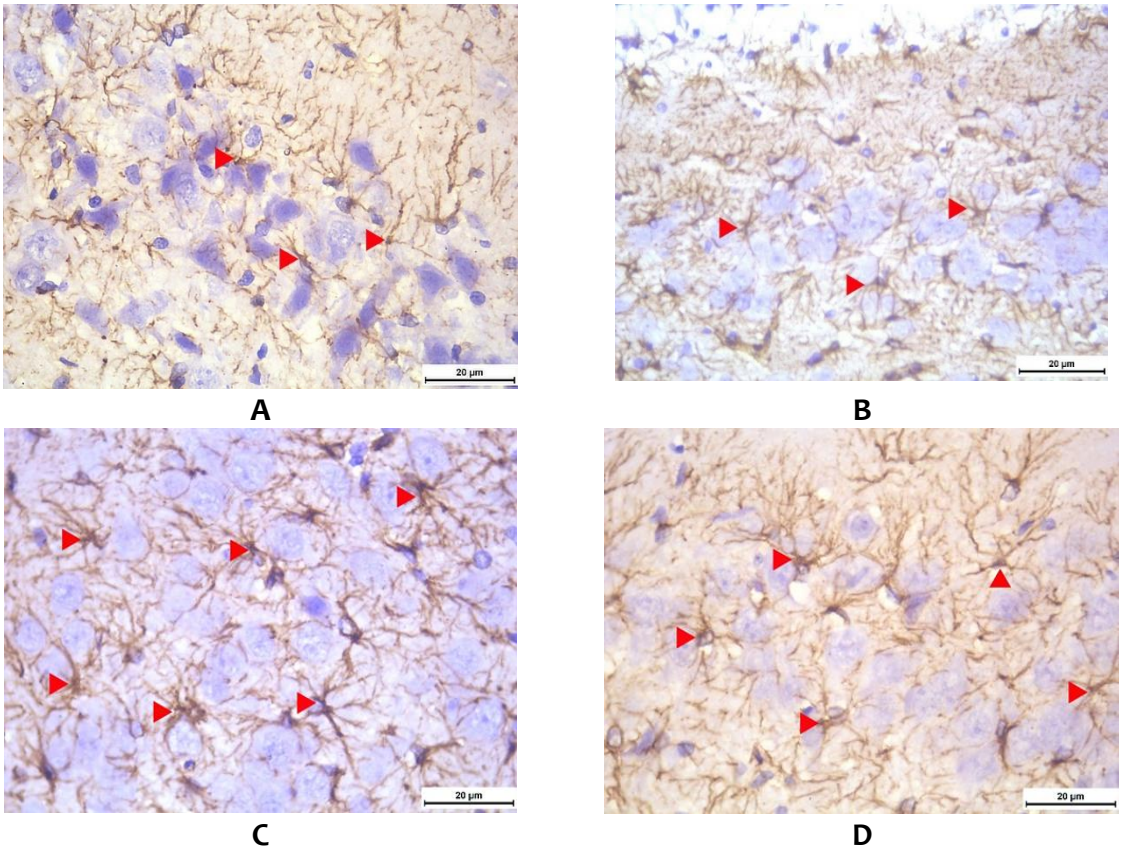

Figure 5: Photomicrographs of the hippocampus CA3 region stained with GFAP X 630 from different groups. **(A)** [Negative Control group] shows weak positive brownish immunoreaction of the astrocytes (arrowheads) in between pyramidal cells. **(B)** [Coenzyme Q 10 group] shows immunoreaction of the astrocytes (arrowheads) similar to the control group. **(C)** [Diet Coke group] shows an increase in the number, processes, and size of positive brownish star-shaped astrocytes (arrowheads) between pyramidal cells. **(D)** [Coenzyme Q10 + Diet Coke group] shows a decrease in the number, processes, and size of positive brownish immunoreactive star-shaped astrocytes (arrowheads) compared to the Diet Coke group.

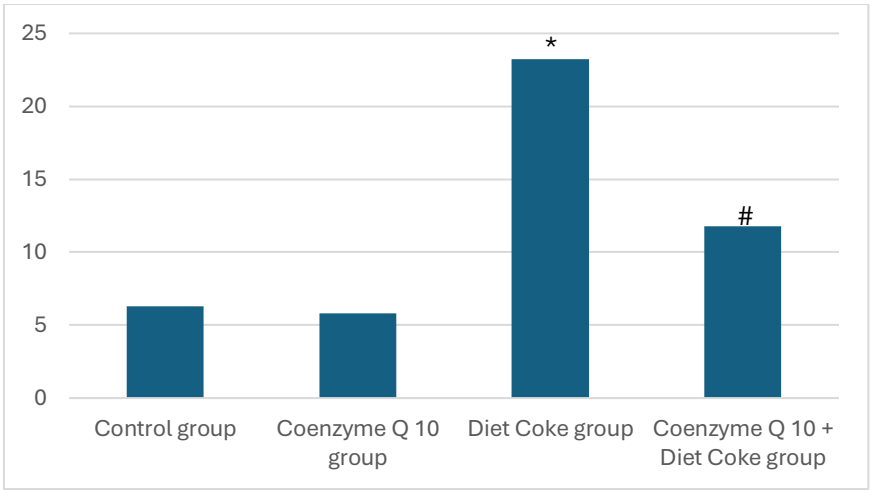

Figure 6: The mean area percentage of GFAP immunostaining in the different groups.
* Statistically significant compared to the control group (P<0.01)
 # Statistically significant compared to the Diet Coke group (P<0.01)

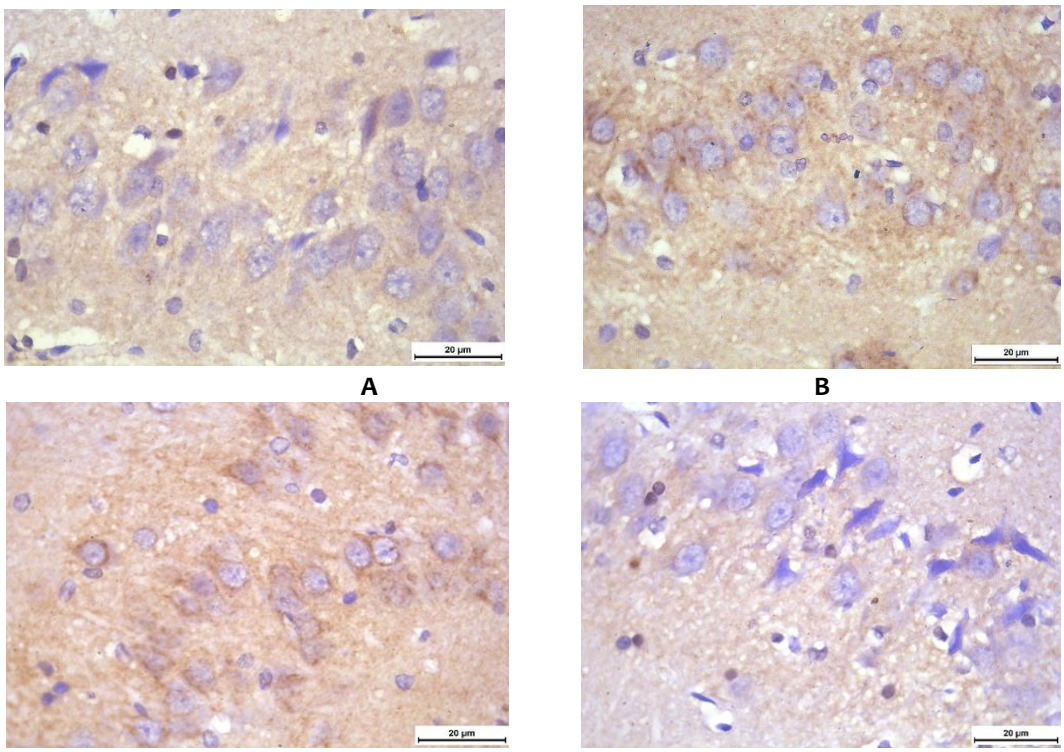

Figure 7: Photomicrographs of the hippocampus CA3 region stained with caspase 3 X 630 from different groups. ***(A)*** *[Negative control group] shows negative brown cytoplasmic immunoreaction of the large pyramidal cells.* ***(B)*** *[Coenzyme Q10] shows immunoreaction more or less similar to that of the control group.* ***(C)*** *[Diet Coke group] shows increased brown cytoplasmic reaction of the large pyramidal cells.* ***(D)*** *[Coenzyme Q10 + Diet Coke] group shows a decreased brown cytoplasmic reaction of the large pyramidal cells compared to the Diet Coke group.*

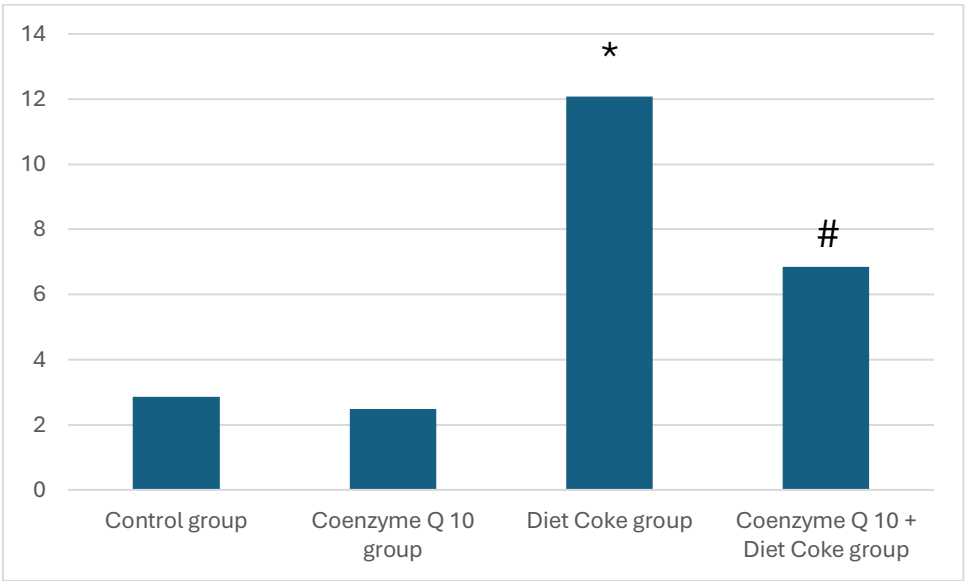

Figure 8: The mean area percent of caspase 3 immunostaining in the different groups.
**Statistically significant compared to the control group (P<0.01)*
 # Statistically significant compared to the Diet Coke group (P<0.01)

Additionally, it exhibits significant histomorphological changes due to oxidative stress in numerous brain regions[18]. This study aims to evaluate the histological alterations caused by a brief diet of coke consumption on the rat hippocampal structure. In the current study, in the Diet Coke group (group III), every rat received 5 ml of Diet Coke, divided equally into two doses every six hours by oral gavage. This dose is documented in this animal model and previously used by Abd El-Wahed *et al.* (2019)[2]. In the current work, light microscopic examination of H&E-stained sections of the hippocampus CA3 region of group III (Diet Coke-treated rats), revealed marked histopathological changes in the form of many degenerated pyramidal cells with no obvious nuclei. These degenerative changes were in accordance with what was found by other researchers who investigated the effect of aspartame on rats' liver[19,20]. These degenerative changes can be clarified based on metabolic pathways of aspartame which could create a state of oxidative stress in tissues. It was mentioned that when aspartame is metabolized in hepatocytes, reactive metabolites are formed, then they are normally handled by renal enzymatic degradation. When aspartame is used excessively, these reactive products cause reactive oxygen species (ROS) to develop. Later, the disparity between the generation of ROS and their removal occurred, leading to oxidative stress, which causes cell damage. These ROS interact with macro-molecules such as proteins, lipids (essential components of nuclear and cellular membranes), and DNA[21]. In the current work, light microscopic examination of Cresyl fast violet stained sections of the hippocampus CA3 region of group III (Diet Coke-treated rats), revealed diminished purple Nissl's granules in the perikarya of pyramidal cells, which was statistically significant compared to the control rats. This was in agreement with Abd El-Wahed *et al.* (2019) who demonstrated the effect of Diet Coke on the rat cerebellar cortex, using a toluidine blue stain and found that the cytoplasm of Purkinje cells of the cerebellum was lightly stained in Diet Coke-treated group, in comparison to control rats, with the apparent decline in the content of their Nissl's granules. They attributed such a reduction to oxidative stress caused by aspartame. The oxidative stress led to neuronal degeneration, which is associated with loss of Nissl's granules; and chromatolysis[2]. This was also confirmed by U-pathi *et al.* (2024) who investigated the potential underlying molecular mechanism of the injurious impact of aspartame taken orally on the rat cerebral cortex. They found that Cresyl fast violet stain of sections of the cerebral cortex of aspartame-treated groups, showed weak Nissl staining, with fewer positive Nissl-stained pyramidal cells, especially with high doses of aspartame. They mentioned that the decreased number of Nissl-stained positive cells was strongly associated with extensive malfunction of mitochondria and might be also correlated with the elevated levels of factors of apoptotic pathway, in the aspartame-treated group, in their study[22]. This is also consistent with our finding, in the current work, related to the increased brown cytoplasmic reaction of the large pyramidal cells of the hippocampus CA3 region stained with caspase 3 immunostaining than that of the control group. The observed increased immunoreaction was confirmed morphometrically and statistically. It's possible that exposure to certain harmful compounds caused programmed cell death, or apoptosis[23]. Even at low dosages, ASP is hazardous, particularly to the neurological system [24]. Numerous pathways have been proposed to explain apoptosis and cell death, including disruptions in the glutathione level that allow the production of neurotoxic reactive species; including nitrogen and oxygen

types[25,26]. U-pathi *et al.* (2024) added that besides induced oxidative stress and depressed antioxidant capacity, the injurious impact of aspartame taken orally on the rats' cerebral cortex could occur through various possible underlying molecular mechanisms, including inhibited mitochondrial biogenesis and activated the expression of apoptosis-related proteins; Caspase-3 and Bcl-2-associated protein x (Bax)[22]. Regarding the PTAH stain of the hippocampus CA3 region of the Diet Coke group, examination revealed decreased dendrite arborizations of the pyramidal cells. This was in accordance with Kamel (2015) who reported that aspartame consumption significantly decreased the level of brain-derived neurotrophic factors in rats' cerebral cortex[27]. Such factors support cell survival, differentiation, proliferation, dendrites branching, and synapse formation in the brain[28]. In the current study, examination of the hippocampus CA3 region stained with GFAP immunostaining, of the Diet Coke group, revealed strong positive cytoplasmic immunoreaction in the astrocytes indicated by increased number, processes, and size of positive brownish star-shaped astrocytes in between the pyramidal cells. The observed increase was statistically significant in comparison to that of the control rats. A similar result was found by other researchers[2, 22, 24, 29]. They found that the expression of GFAP in aspartame-treated groups was significantly increased ($p < 0.01$) when compared to that of the control group. GFAP expression is gradually upregulated by astrocytes in response to neuronal injury. Accordingly, the Reactivity of GFAP can be utilized as a reliable and sensitive marker for neurological injuries[30]. Onaolapo, Onaolapo, and Nwoha (2016) found that chronic administration of excessive amounts of aspartame, in mice, could enhance cerebral oxidative stress and reactivity of GFAP in the cerebral cortex[24]. Additionally, it was reported that long-term aspartame consumption may cause dose-dependent damage to neuronal cells of the hippocampus and an increase in glial cell density [29]. It was proposed that a rise in the astrocytic count, also known as reactive astrogliosis, could compensate for aspartame-induced damage to the central nervous system, which could explain this[26]. U-pathi *et al.* (2024) added that aspartame can modify pathways of cell signaling associated with oxidative stress in neuroglial cells and neurons of the rat cerebral cortex[22]. Bagheri *et al.* (2023) reported that many recorded agents such as Coenzyme Q10 possess neuroprotective properties to guard against neuronal impairment[31]. In the current research, microscopic light and immunohistochemical examination of (Coenzyme Q 10 + Diet Coke group) showed that Coenzyme Q10 protected the neurons from being degenerated by Diet Coke. Examination of H & E-stained sections showed normal pyramidal cells (P) with vesicular nuclei and prominent nucleoli. This was in agreement with other earlier studies. S Yousef *et al.* (2019) investigated the neuroprotective effect of Coenzyme Q 10 against lead acetate neurotoxicity in male Wister albino rats and found that treatment with Coenzyme Q 10 could alleviate markedly the cortical tissue pathological alterations, produced by lead acetate[32]. Badawy Khair and Mohammed (2021) found most Purkinje and granular cells of the cerebellar cortex, of the coenzyme Q10-treated group, had a similar appearance to that of the control group, when atherosclerosis induced in experimental rats[33]. Alnuimi and Alabdaly (2022) investigated the role of Coenzyme Q10 against copper sulfate toxicity in the chick's models. They observed that the cerebral cortex of the group treated with Coenzyme Q10 showed a normal appearance of neurons and glial cells,

away from a few histopathological changes[34]. Additionally, Cresyl fast violet stained sections of (Coenzyme Q10 + Diet Coke), showed pyramidal cells perikarya with increased purple Nissl's granules, in comparison to the Diet Coke group. This was confirmed morphometrically and statistically. This was in line with Vittalrao *et al.* (2023) who studied the cognitive enhancing activity of Coenzyme Q 10, either alone or in combination with antihypertensive Ramipril, on sleep deprivation-induced cognition impairment. They observed that in Coenzyme-administered rats, there was a discernible increase in healthy neurons and a significant decrease in the degenerating pyramidal neuronal cell bodies, hippocampus CA3 region. They concluded that Coenzyme Q10 alone or combined with Ramipril, was able to improve memory and learning against cognition impairment induced by acute sleep deprivation[35]. This also could explain the protection of dendrite arborizations of the pyramidal cells detected by PTAH stain of the hippocampus CA3 region of the (Coenzyme Q10 + Diet Coke) group in the current research. This can be supported by Inoue *et al.* (2023) who suggested that Coenzyme Q10 could protect middle-aged mice's motor ability by activating the function of brain mitochondria and the excitatory postsynaptic potential amplitude level in the basal field in the motor cortex pathways. This occurred probably through increasing the efficacy of synaptic plasticity between such arborizations[36]. Regarding GFAP immunostaining of the hippocampus CA3 region of (Coenzyme Q10 + Diet Coke) group, in the present work, there was a diminution in the number, processes, and size of positive brownish immunoreactive star-shaped astrocytes, compared to that of the Diet Coke group. This was confirmed morphometrically and statistically. This was in accordance with Badawy Khair and Mohammed (2021) who reported that GFAP immunostaining of astrocytes in the Coenzyme Q10 treated group, showed decreased positive staining yielding moderate positive immune reaction in the cerebellar cortex of the atherosclerotic model[33]. In the current study, examination of sections stained with caspase 3 immunostaining, showed decreased brown cytoplasmic reaction of the large pyramidal cells, compared to the Diet Coke group. This was in agreement with S Yousef *et al.* (2019) who observed that Coenzyme Q10 significantly ($P < 0.05$) decreased the expression of proapoptotic and augmented the anti-apoptotic proteins expression in rat cortical tissues when co-administered with lead acetate in induced neurotoxicity model[32]. This was also confirmed by Gel *et al.* (2024) who concluded that Coenzyme Q 10 combined with ozone therapy could provide a neuroprotective effect in spinal cord traumatic injury, based on the significant decrease of tissue caspase 3 ($p= 0.001$) and improvement of the histopathological, ultrastructural and neurological outcomes in rats receiving such a treatment[37]. It was mentioned that Coenzyme Q10 participated crucially in the production of energy and mitochondrial maintenance as it could hinder the splitting of mitochondria and enhance mitochondrial dynamics through diminishing fission protein 1 (Fis1) and dynamin-related protein 1 (Drp1). Both are essential protein fission markers that can control mitochondrial dynamics[38]. Moreover, Coenzyme Q10 is a powerful antioxidant and can reduce oxidative stress providing an antioxidant status with restoration of the balance between oxidants and antioxidants (by upregulation of their gene expression) in the cerebral cortex of experimental animals. This can occur due to its ability to significantly increase the total antioxidant capacity (TAC) level, scavenge reactive oxygen species (ROS), and decrease lipid peroxidation, particularly

peroxide radicals, thus guarding cell membranes against free radicals- induced oxidative damage[32, 35, 36, 39]. Consequently, Coenzyme Q10 was reported to prevent programmed cell death as an antiapoptotic agent that could modulate apoptotic proteins via its antioxidant activity which is considered an important mechanism through which CoQ10 exerts its neuroprotective effects[31, 40, 41]. Furthermore, it was suggested that Coenzyme Q10 had an anti-inflammatory effect as it could modify the way that genes implicated in the inflammatory process are expressed, causing a reduction of the elevated cortical levels of tumor necrosis factor α (TNF- α) and interleukin 1β (IL-1β) and downregulation of their gene expression[32].

Conclusion

According to the histopathological and immunohistochemical results of the present research, Coenzyme Q 10 has an ameliorative role in attenuating histopathological changes in the hippocampus induced by Diet Coke consumption in adult male albino rats.

Recommendation

The authors recommend further clinical trials to confirm the ameliorative effect of Coenzyme Q 10 in attenuating Diet Coke's harmful effects to avoid such effects.

Conflict of Interest

There were no declared conflicts of interest by the authors.

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