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Assessment of the Potential Toxic Consequences of Energy Drinks on the Brain Tissue of Maternal Wister Rats Throughout Gestation and Lactation

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Abstract

BRAIN tissue is the most vulnerable tissue to toxicity especially in the gestation and lactation period and the most consumed beverages between all ages is energy drinks (EDs). The objective of this study is the evaluation of the possible toxicity of EDs ingestion in the brain tissue of maternal rats during gestation and lactation, as more is needed to be reported confirming its effect on these critical periods until now. A total of eighteen female Wistar rats were categorized into three groups. From the fifth day of pregnancy until the end of lactation, which lasted for 38 days, the groups were given daily doses of 3.57 and 7.14 ml/kg of body weight in the low and high dose groups, respectively. The control group received a saline solution. The ED-treated groups exhibited decreased acetylcholinesterase (AchE) levels and increased dopamine levels. Additionally, the brain tissue exhibited histopathological alternation and the initiation of oxidative stress. This was evidenced by an elevation in malondialdehyde levels and a disruption in the synthesis of antioxidant enzymes. Consequently, there was a pronounced degradation of DNA damage in the brain. EDs impacted the neurotransmitters and AchE in maternal rats, leading to an increase in the generation of free radicals. Consequently, this process stimulated the destruction of brain cells through oxidative stress. As a result, it had a negative impact on the cellular and genetic well-being of the maternal brain.

Keywords: Energy drinks, brain tissues, oxidative stress, DNA damage.

Introduction

Since the emergence of the COVID-19 epidemic, there has been a shift in the beverage market, with individuals increasingly purchasing caffeinated beverages with higher sugar content, such as energy drinks (EDs). In response to the pandemic, individuals resorted to using various cognitive enhancers to alleviate stress and boredom while engaging in gaming and screen activities [1]. Therefore, global youth generated approximately $61 trillion in profits from their purchases by 2021. This was due to the prolonged wakefulness and enhanced attention they experienced from consuming products containing caffeine, a psychoactive stimulant for the brain [2, 3]. Regrettably, adolescents constitute the predominant demographic endorsing these beverages in Egypt, with approximately 14.2% consuming them multiple times daily regularly [4]. The prevalence of unintentional intake of energy drinks in Egypt can be attributed to the need to combat fatigue and enhance focus, with 46.4% and 28.7%, respectively [4].

Energy drinks typically include approximately 70-200 mg of caffeine per 500 ml cans, which enhances cognitive function [3]. Regrettably, the excessive administration of them resulted in significant adverse consequences, such as low blood pressure, high blood pressure, and seizures [5]. Other often utilized components in energy drinks include taurine, sugar, vitamins, and ginseng in the form of herbal extracts. Taurine is classified as a non-essential amino acid. The recommended daily intake of taurine ranges from 40 to 400 mg. However, energy drinks typically include significantly higher amounts, approximately 750 to 1,000 mg per serving [6]. High levels of taurine exposure have been found to have significant harmful consequences in children, as Rubio *et al*. [7] reported. The predominant sugars in energy drinks (EDs) are sucrose and fructose. Excessive consumption of these sugars has been

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shown to affect brain function negatively and is strongly associated with alterations in mood, particularly depression, and happiness [8, 9]. Regular exposure to EDs in the brain can lead to sleeplessness and neuronal damage [4, 6].

The brain tissue is the most intricate organ in the body, as it connects all organs through billions of nerve cells that establish synapses and connections [10]. Consequently, it regulates cognitive processes, sensory perception, motor function, memory, communication, and reaction to diverse environmental stimuli [11]. Proper nutrition is crucial for optimal development, particularly during the gestation and lactation stages. This is because it has a long-lasting impact on both the mother and the infant's brain throughout their lives [2]. Prior research has demonstrated that adequate maternal nutrition throughout pregnancy and breastfeeding significantly influences the long-term health of both the mother and the baby. This is supported by a strong association between nutrition during gestation and lactation and the health outcomes of both the mother and the infant [12]. Appropriate maternal nutrition can prevent numerous disorders in both the mother and their offspring, notably in the brain tissue.

Unfortunately, there are not enough papers discussing the impact of EDs on the brain tissue of the most sensitive time in the adult female lifespan, pregnancy, and lactation. Thus, the current work highlights the effects of ED consumption at these times in the brain tissue by measuring the acetylcholine esterase and dopamine levels. Moreover, this study aims to assess oxidative status, DNA damage, and histopathology in the brain tissue.

Material and Methods

Chemicals

Sigma-Aldrich supplied the AChE, while the dopamine ELISA kits were purchased from Abcam Company (Cambridge, UK). The reagent kits of oxidative stress markers were obtained from the Biodiagnostic Company (Giza, Egypt). EDs were supplied from the local market in Cairo, Egypt. Energy drinks (EDs) were taken from the local market.

Animals and work design

Eighteen Wistar female rats were purchased from the animal house of the Veterinary Medicine at Cairo University weighing (170-200) grams. They were housed in plastic cages with stainless steel covers (3 rats/cage) under controlled temperature, humidity, and lighting conditions. There are three groups, with six rats in each one. From the fifth day of pregnancy, rats were supplied orally 3.57ml/Kg b. wt. was a low dose group and 7.14 ml/Kg b. wt. Treated rats were a high-dose group. At the same time, the control group took distilled water.

Tissue preparation

The brain tissue extracted from the rats was immersed in a phosphate-buffered solution (PBS) to eliminate any remnants of blood. The collected tissue was divided to determine the levels of acetylcholine esterase (AChE), dopamine as a neurotransmitter, oxidative biomarkers, comet assay to assess the damage to the intact DNA, and histological alterations.

Estimation of acetylcholine esterase (AChE)

The tissue was homogenized in 0.1 M phosphate buffer, then centrifuged for 5 minutes at 14000 rpm to separate and take the supernatants for assay and put the fresh working reagent to assay AchE [13].

Determination of Dopamine level

The competitive ELISA kit used the microtiter plate format to quantitatively measure the dopamine level in the brain tissue according to the manufacturer's guidelines and instructions.

Oxidative biomarkers determination:

Oxidative biomarkers GSH, MDA, and SOD in the brain tissue of the EDs-supplied rats and control rats were accessed by using the colorimetric methods [14-16] with (CAT. No. GSH 25 17), (CAT. No. MDA 25 29), and (CAT. No. SOD 25 11), respectively.

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Comet Assay

It is an alkaline method to examine DNA breakage in the nucleus of the maternal brain tissue DNA using a fluorescent microscope (Carl et al. with epifluorescence using filter 15 BP546/12, FT580, and LP590) to analyze slides. The photos of the run fragmented DNA in the electric field from different groups were taken by immediate image capture and scoring of 50 cells with 400 magnification sing Comet 5 image analysis software (Kinetic Imaging, Ltd.). The comet processes were previously

discussed [17-19]. The olive tail moment (OTM) parameter is the most important of comet parameters as it can evaluate and quantify DNA breakage, and it depends on DNA movement and DNA quantity in the tail and its value was calculated using the following equation (1) [20].

$$OTM = \text{tail moment} \times \text{tail} /100. \quad (1)$$

Histological Observation

The collected brains from different rats were put in a fixation solution of 10% neutral formalin saline (pH = 7.0) for 48 hours, fixed in paraffin wax, then cut the forming paraffin wax blocks into 4–6 μm slices and stained by using hematoxylin and eosin to check brain tissues [21]. The pathologist examined the prepared sample blocks under the light microscope and took about ten photos of each examined part.

Statistical Analysis

The Mean (μ) of tests in all studied groups (n = 6) and the standard deviation were measured using SPSS Statistics version 25, then represented as μ ± SD. The variation comparison between studies was carried out using ANOVA and post- hoc LSD with a significant value ($P < 0.05$).

Results

Effect of ED administration on the Acetylcholine esterase and Dopamine

The levels of AChE and dopamine were measured, and the findings are presented in Table 1. There was a statistically significant decrease ($P < 0.05$) in the mean concentration of AChE in the group that received low and high doses of EDs compared to the control group. Simultaneously, the treated groups exhibited a substantial increase in dopamine levels compared to the control group. The low-dose group showed a significant difference ($P < 0.05$) compared to the high dose in levels of AChE and dopamine.

The observation of the brain tissue oxidative biomarkers

The administration of EDs led to a notable rise ($P < 0.05$) in the levels of brain MDA and a substantial decrease ($P < 0.05$) in the levels of brain SOD and GSH in the treated groups of rats, as compared to the control group. Furthermore, there is a significant difference between the low and high-dosage groups (Table 1).

The measurement of the brain DNA damage using comet assay

The comet indicators used to assess DNA fragmentation in brain tissue revealed intact cell structures with no evidence of DNA damage, including an intact head and the absence of a tail in the control group (Figure 1). Nevertheless, both groups supplied by EDs confirmed a substantial increase ($P < 0.05$) in the overall comet indicators of DNA, as presented in (Figure 2).

Histological Observation

Under microscopic examination using H&E staining, the brain of the control rats exhibits regular histological sections, as seen and summarized in Figure 3-4. The histological pattern of the cerebral cortex revealed the characteristic uniform arrangement of neurons, microglia cells, and pyramidal cells in their usual forms. Furthermore, the interstitial substance between nerve cells is often filled with homogeneous neuropil. The pia mater is regularly connected to the well-structured prefrontal cortex layers, including molecular, external pyramidal, and granular layers. These layers have small neuronal cell bodies with rounded nuclei and limited cytoplasm. Standard blood capillaries are present in the acidophilic neuropil, as depicted in (Figure 3). Moreover, it is observed that the pyramidal cell layer and molecular layer of the hippocampus appear to be in a healthy condition. Pyramidal neurons in the pyramidal cell layer possess compact cell bodies characterized by vesicular nuclei and limited cytoplasm. These neurons are densely packed and have a well-organized structure. Glial cells are present in the molecular layer. The granule cell layer of the dentate gyrus and the polymorphic layer were well delineated. The granule cell layer exhibits the clustering of granule cell bodies, which vary in shape from spherical to oval and contain immature neurons (Figure 4).

The rats treated with a low dose of 0.7 ml/200g had various histological abnormalities, as depicted in Figure 5-6. The sample exhibited a minor level of neuropil vacuolation, reduced neurons, and dark-stained pyknotic nuclei. Additionally, there was a moderate level of dilated blood vessels in the layers of the prefrontal cortex and congested pia mater. Figure 4 illustrates the existence of vacuoles and ghost-degraded neurons. Figure 5 shows that the pyramidal neuron cell bodies in the hippocampus need to be more organized and tightly packed. They seem dark and wrinkled and have condensed nuclei with halos around them. Additionally, vacuoles and congestion are observed. The granular cell layer of the dentate gyrus and the polymorphic layer exhibited comparable levels of disruption.

On the other hand, the group that received the highest dose showed a diverse range of histological defects, as depicted in Figure 7-8. Figure 7 shows a significant presence of neuropil vacuolation, which is of moderate degree. Moreover, the extent of blood

vessel expansion and congestion of the pia mater in the layers of the prefrontal cortex. Additionally, one may see black, contracted neuronal cell bodies and highly colored pyknotic nuclei surrounded by pericellular haloes. Simultaneously, the cell bodies of the pyramidal neurons exhibit aberrant characteristics, including disarray, darkness, and shrinkage.

It also includes pyknotic nuclei encircled by pericellular haloes and degenerative neurons. There is a comparable lack of organization in the granular cell layer of the dentate gyrus. Neuropil exhibiting vacuolation.

Discussion

EDs have spread widely among people to do their routine work daily without noticing their adverse consequences on brain tissue health including neuro-transmitters, oxidative stress, morphology, and genotoxicity, especially in the gestation and lactation times. Thus, the present study wanted to shed light on the link between their exposure and their impact on brain tissue in adult pregnant and lactated female rats.

The obtained study measured neuro-transmitter dopamine and AChE which break down neurotransmitter acetylcholine (ACh). AChE enzyme reduction caused the elevation of ACh accumulation in muscarinic and nicotinic toxicity including parasympathetic, sympathetic, and skeletal muscle signs and symptoms [22]. The current research found a significant reduction in the AChE level of the EDs administrated in high and low-dosage rats compared to control rats.

The reduction alteration of AChE activity in the brain tissue of both EDs-treated groups aligns with recent research conducted by Stoytcheva *et al*. [23], Okello et al. [24], and Hanna et al. [25]. Another study by Ebuehi *et al*. [26] revealed that EDs supply caused a decline in AChE activity. The cause of these changes was the administration of caffeine, which induced a decrease in the activity of AChE as described by Fabiani *et al*. [27]. Moreover, Pohanka and Dobes [28] reported that caffeine, the primary component in energy drinks, contributes to AChE inhibition, as a specific noncompetitive inhibitor by binding to Trp86 in AChE through π–π interaction, Ser125, and Tyr133with hydrogen bond. Based on study of Attia and Nasr [29] demonstrated that cytotoxicity in the brain tissue of rats may be due to the reduction of AChE boosted the accumulation of acetylcholine.

The other neurotransmitter is dopamine which is known as the pleasure hormone, as it controls emotion. The elevation of dopamine levels in the brain refers to drug abuse [30]. The EDs supplied maternal rats in the present study induced dopamine secretion in the brain tissue compared to the control.

Previous researchers indicated that long-term consumption of EDs leads to an increase in dopamine levels release in hippocampus and cerebral cortex, as demonstrated by Owolabi *et al*. [31] and Volkow *et al*. [32]. The study by Vargiu *et al*. [33] stated that high levels of dopamine release in administrated EDs occur in drug additives, and it is due to the three main ingredients of EDs caffeine, sugar, and taurine. Nevertheless, the research conducted by Bawazir [34] showed that prolonged consumption of EDs resulted in a notable reduction in dopamine levels in the brain. This decrease in dopamine content within cells is attributed to the ongoing dopamine release from nerve cells.

The first explanation for dopamine elevation in the brain tissue is owing to caffeine, which primarily acts as an antagonist to adenosine A2A receptors and an agonist to dopamine receptors, exhibiting psychostimulant effects. Adenosine A2A receptors suppress the activity of dopamine D2 receptors by reducing their binding strength and interfering with their signal transmission. The antagonistic relationship arises from allosteric receptor-receptor interactions within A2AR/D2R heteromeric complexes [35]. Another factor may be contributing to dopamine elevation is the high sugar content in EDs, which results in the impairment of prefrontal control, a crucial factor in regulating behavior. This impairment occurred because of the downregulation of D2R in the striatum, which regulates the prefrontal cortex. As a result, there is an increase in the release of dopamine in the dorsal and ventral striatum, while the activity in the prefrontal regions decreases. Thus, it led to behavioral abnormalities and a lack of self-control due to an unconscious increase in consumption.

There is a strong correlation between the primary components of EDs (sugar and caffeine) and dopamine, a neurotransmitter associated with pleasure. Consuming large amounts of sugar and caffeine prompts the brain to release dopamine, similar to the mechanism observed in drug addictions. That may explain why individuals constantly crave these beverages as demonstrated by prior studies by Volkow *et al*. [32] and (Chen and Schwarzschild) [36]. There was another direct link between high sugar consumption, oxidative stress, and abnormal dopamine levels, as described by Juárez *et al*. [30], who found evidence that abnormal dopamine levels in the brain tissue are caused by oxidative stress.

The present investigation demonstrated a notable increase in the level of the oxidative stress marker MDA and a considerable decrease in the levels of the

antioxidant defense enzymes (GSH and SOD) in the brain tissue of rats treated with EDs. MDA is a general indicator of oxidative stress since it can evaluate lipid peroxidation resulting from oxidative stress. Antioxidants SOD and GSH serve as a defensive mechanism against free radicals by neutralizing them and inhibiting their ongoing generation [37]. Therefore, an increase in MDA level and a decrease in SOD and GSH indicate the presence of oxidative stress.

Several studies have shown that consuming excessive amounts of EDs leads to a notable rise in MDA levels and a considerable decrease in antioxidant defences in brain tissue [25, 38, 39]. An additional study conducted by Olofinnade *et al*. [40] revealed that introducing sodium benzoate as a beverage preservative in EDs leads to oxidative stress in brain tissue, intensifying the inflammatory reaction in the brain.

The most severe toxicological effect associated with oxidative stress was observed at the level of DNA integrity. Results of the present study demonstrated a significant rise in DNA degeneration in the brain tissue of maternal rats exposed to EDs.

The highly adverse impact of oxidative stress to the brain DNA in the EDs exposed rats in the obtained work is due to the bad antioxidant defence. This observation is aligning with many authors [41-43], they stated that combining sugar and sodium benzoate as preservative in EDs leads to oxidative stress in brain tissue. This stress triggers a reaction with proteins, DNA bases, and oxidized lipids, forming advanced glycation end-products (AGEs). These AGEs can cause instability and deterioration of lipid peroxidation by-products.

A further impact of oxidative stress is the impairment of vital cellular components and their structural integrity. The study revealed significant damage to several areas of the brain tissue in ED-treated groups. The group that received EDs exhibited various histological abnormalities, such as neuropil vacuolation, reduced neuronal count, and dilatation of blood vessels in the pia matter and prefrontal cortex layers. The hippocampal portion exhibited disarrayed and sparsely arranged cell bodies of pyramidal neurons, characterized by their darkened appearance.

The current finding aligns with a prior investigation conducted by Abdelwahab *et al*. [44], which observed the presence of congested blood vessels, dark nuclei, and cellular harm in brain tissue and all these changes in brain histopathology and neuron degenerative could be contributed with the bad of antioxidant status defence and intact DNA damage caused by caffeine and preservatives in EDs. Previous research conducted by Bawazir [34] and Salih *et al*. [45] corroborated the findings of this study about the reduction in the number of nerves and neurons in the hippocampus, as well as the presence of neurons in the cerebral cortex that exhibit characteristics such as a deep stain, shrinkage, and small nuclei and these changes may be due to the caffeine in EDs.

There was a direct correlation between abuse of psychostimulants, oxidative stress, and neurodegenerative diseases, and there was an indirect correlation between AChE and neurodegenerative diseases [23, 46]. Thus, the reduction of AChE and elevation of dopamine level in the brain is related to oxidative stress induction, which leads to DNA intact damage and neuron damage, as shown in the histopathology in the obtained study. Thus, EDs motivate the risk of getting neurodegenerative diseases.

Conclusion

The brain tissue of maternal rats who received EDs exhibited severe damage characterized by the induction of oxidative stress, impairment of intact DNA, histopathological changes, and reduced AChE production. Moreover, the disaster is caused by energy drinks impacting the dopamine level, influencing people's emotions and tendency to consume these beverages without adopting caution. Hence, it is imperative to disseminate knowledge about the potential hazards and illnesses across all age groups, with a particular focus on pregnant and nursing women, to prevent any issues in their well-being and the well-being of their infants. Additional research is required to assess the potential adverse effects on the brain tissue of newborns whose mothers were exposed to EDs during pregnancy and breastfeeding. This is necessary to prevent issues in future generations.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Science, Cairo University, Egypt (ethics approval number; CU I F 72 22).

TABLE 1. Alternation of the AChE and dopamine and oxidative biomarkers by EDs administration in the brain tissue

Groups Parameters	Control	Low Dose	High Dose
AChE (ng/mL)	17.79 ± 0.53	15.18 ± 0.85[a]	13.42 ± 0.40[a,b]
Dopamine (ng/mL)	123.28 ± 4.21	134.6 ± 5.63[a]	153.33 ± 5.63[a,b]
GSH (mg/g tissue)	1.17± 0.26	0.85 ± 0.1[a]	0.60 ± 0.16[a,b]
MDA (nmol/g. tissue)	16.39 ± 0.59	33.16 ± 1.88[a]	39.42 ± 2.71[a,b]
SOD (U/g. tissue)	25.22± 0.90	17.11± 1.14[a]	15.78 ± 0.99[a,b]

Note: The data are represented as Mean ± SD (n = 6), and the lowercase letters revealed a statistically LSD significant (P < 0.05) variation:
(a) Significant compared with control.
(b) Significant compared with low dose treated group.

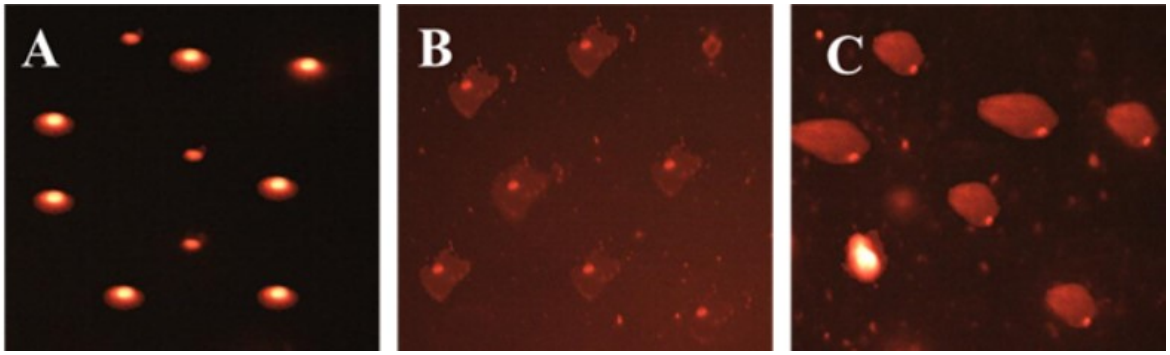

Fig. 1. Fluorescent microscope pictures for the effect of energy drinks on the nucleus of A: Control group, B: Low dose group (3.57ml/kg) and C: High dose group (7.14 ml/Kg) in the brain tissue of maternal rats.

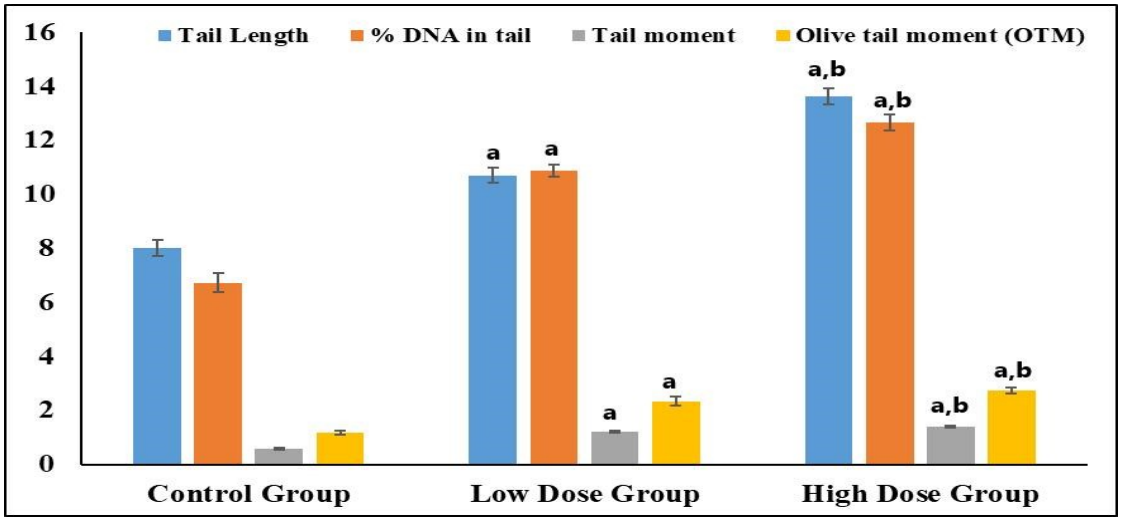

Fig. 2. Effect of energy drinks on the comet assay parameters of the brain tissue in the studied groups.
Note: The data are represented as Mean ± SD (n = 6), and the lowercase letters revealed a statistically LSD significant (P < 0.05) variation:
(a) Significant compared with control.
(b) Significant compared with low dose treated group.

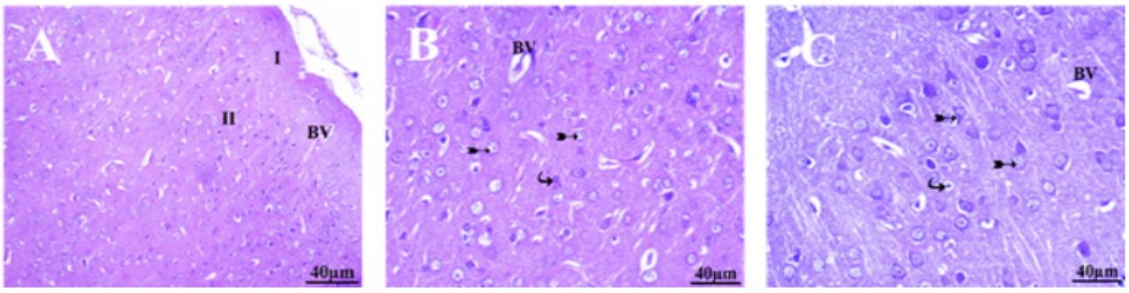

Fig. 3. A-C: Photomicrographs of the control dose rats' brains (H&E-stained). Presence of the typical shape of the brain microglia cells (curved arrow), pyramidal cells (bifid arrow), the monomorphic pattern of neurons, and typical occupation of uniform neuropil. The attached organized layers between Pia matter and the prefrontal cortex are molecular (I) and external pyramidal and granular (II), which are ordinary. Blood capillaries (BV) are in good shape within the acidophilic neuropil.

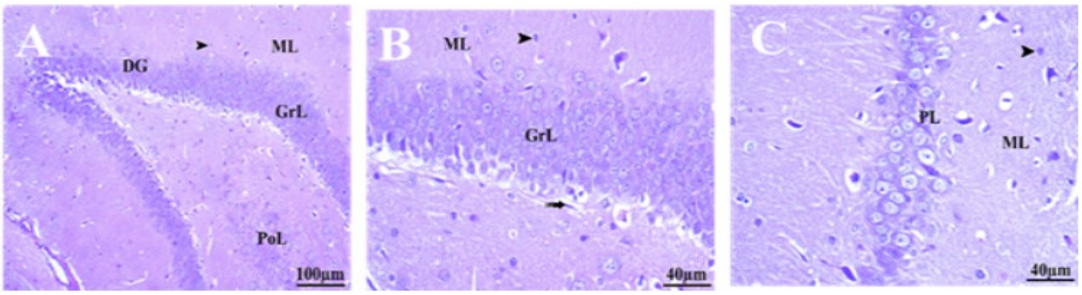

Fig. 4. (A-C): Photomicrographs of the control dose rats' brain (H&E-stained). There was a normal hippocampus's pyramidal cell layer (PL), molecular layer (ML), and glial cells (arrowhead) in ML. The granule cell layer (GrL) is well-defined by the dentate gyrus (DG) and polymorphic layer (PoL). The aggregation of granule cell bodies (dotted arrow).

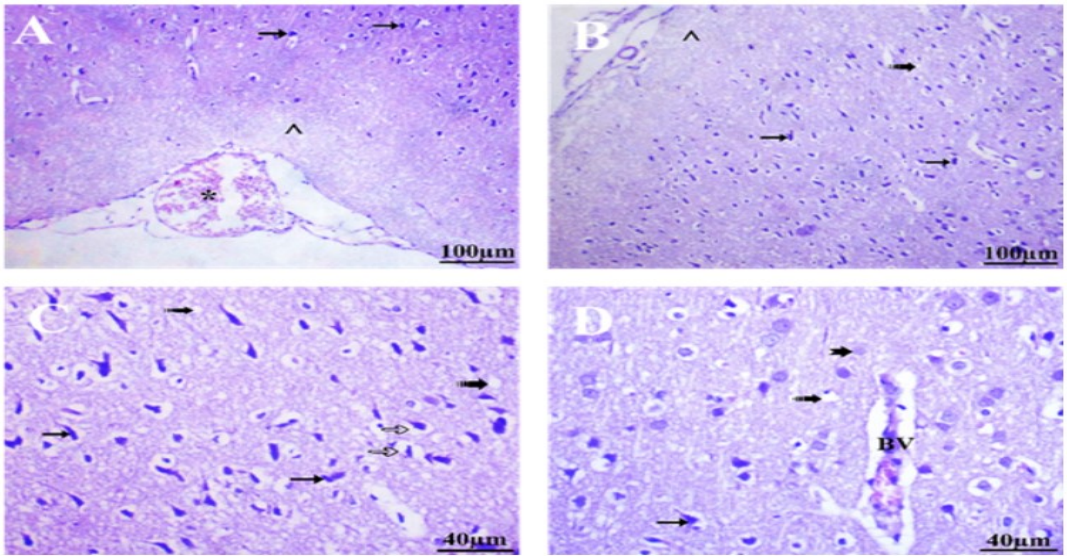

Fig. 5. (A-D): Sections of the low-dose rats' brains (H&E-stained). It appeared to have some histopathological abnormalities. Neuropil vacuolation (^), dark stained pyknotic nuclei (arrow), prefrontal cortex (BV), congested pia matter (*), vacuoles (dotted arrow), and ghost degenerated neurons (notch arrow).

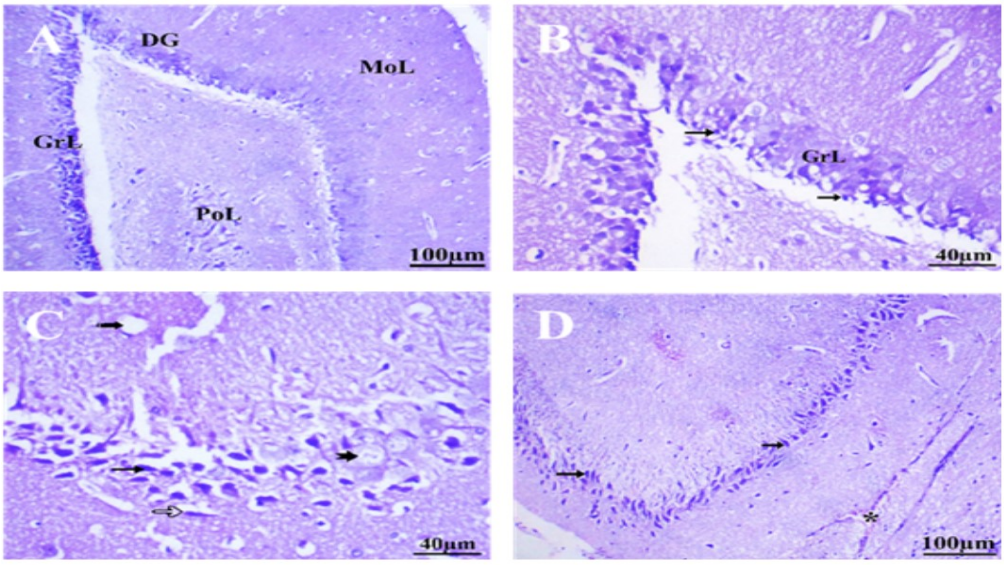

Fig. 6. (A-D): Photomicrographs of the low-dose rats' brain sections (H&E-stained). Presence of some histopathological abnormalities such as abnormal pyramidal neuron cell bodies, vacuoles and congestion. Also, the granular cell layer of the dentate gyrus and polymorphic layer are disordered. Pyknotic nuclei (arrow), pericellular haloes (hollow arrow), vacuoles (dotted arrow), congestion (*), The granular cell layer (GrL), dentate gyrus (DG) and polymorphic layer (PoL).

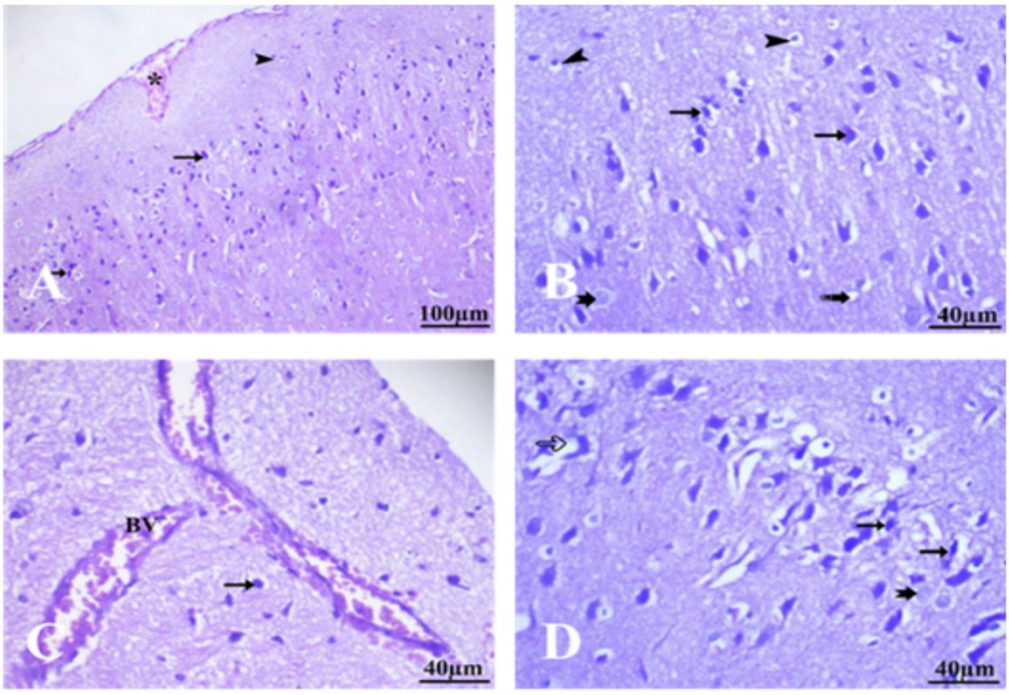

Fig. 7. (A-D): Photomicrographs of the high-dose rats' brain sections (H&E-stained). Display a wide range of histopathological abnormalities, including neuropil vacuolation (dotted arrow), blood vessel dilation (BV) and pia mater congestion (*). The pericellular haloes (hollow arrow) have dark, shrunken neuronal cell bodies and highly pigmented pyknotic nuclei (arrow).

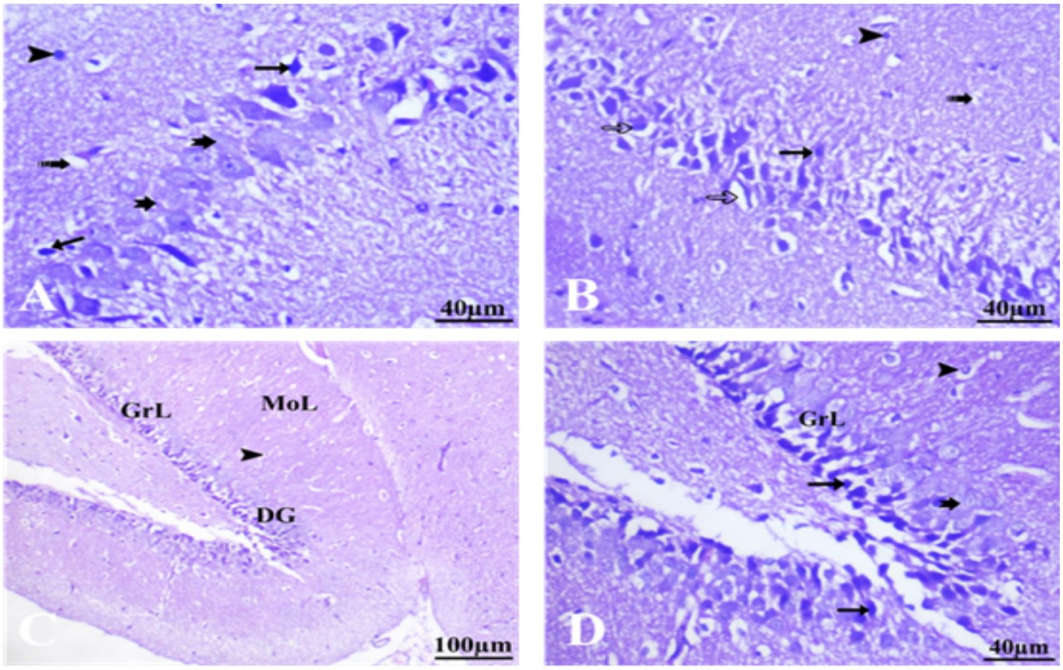

Fig. 8. (A-D): Photomicrographs of the high-dose rats' brain hippocampus sections (H&E-stained). The cell bodies of pyramidal neurons in the hippocampus are abnormal as they are disorganized, dark, and shrunken. Also, it contains pyknotic nuclei (arrow), which are surrounded by pericellular haloes (hollow arrow) and degenerated neurons (ghost cells) (notch arrow). There is similar disorganization in the granular cell layer (GCL) from the dentate gyrus. Vacuolation of neuropil (doted arrow).

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تقييم الآثار السامة المحتملة لمشروبات الطاقة على أنسجة المخ لدى أمهات جرذان ويستار خلال فترة الحمل والرضاعة

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الملخص

تعتبر انسجة المخ الاكثرعرضة للتسمم خاصة فى فترة الحمل والرضاعة وان اكثر المشروبات تداولا بين جميع الفئات العمرية هى مشروبات الطاقة. لذا هذا العمل يهدف الى تقييم السمية المحتملة لتناول مشروبات الطاقة على انسجة مخ اناث جرذان ويستار خلال فترتى الحمل والرضاعة بسبب الحاجة الماسة للتقارير التى تؤكد تأثيرها خلال تلك الفترات المهمة. تم استخدام اجمالى ثمانية عشر فأر من اناث جرذان ويستار وتقسيمهم إلى ثلاث مجموعات. استمر اعطاء جرعات يومية لتلك المجموعات لمدة 38 يومًا بداية من اليوم الخامس من الحمل حتى نهاية الرضاعة حيث تلقت مجموعتى الجرعات المنخفضة والعالية حوالى 3.57 و7.14 مل/كجم من وزن الجسم من مشروبات الطاقة بينما تلقت مجموعة التحكم محلول ملحي. أظهرت النتائج أن المجموعات المعالجة بمشروبات الطاقة انخفاض واضح فى مستويات الأسيتيل كولين استريز (AchE) مع زيادة فى مستويات الدوبامين. كما اظهرت تغيرات نسيجية مرضية على انسجة المخ مع الإجهاد التأكسدي . وقد ثبت ذلك من خلال ارتفاع مستويات مالونديالدهيد مع اضطراب في تخليق إنزيمات مضادات الأكسدة مما أدى الى تدهور واضح في الحمض النووي في المخ. نستطيع القول أن مشروبات الطاقة اثرت على النواقل العصبية لدى الأمهات مما أدى الى زيادة الجزيئات الحرة والتى حفزت تدمير أنسجة المخ عن طريق الإجهاد التأكسدي الذى كان له تأثير سلبى على الصحة الخلوية والوراثية لدماغ الأمهات.

الكلمات الدالة: مشروبات الطاقة ، انسخة المخ ، الاجهاد التأكسدى ، تلف الحمض النووي.