Original Article

Biochemical, Toxicological, and Histological Changes of Energy Drinks on Brain and Pancreatic Tissues in Pregnant Wistar Rats

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ABSTRACT

Introduction: On a global scale, the intake of energy drinks has been consistently rising. The chemical composition of energy drinks can lead to various adverse effects, such as neurological symptoms like tremors and restlessness, heart symptoms like palpitations or tachycardia, and occasionally severe gastrointestinal symptoms.

Aim of the Work: The aim of this study was to assess the potential adverse impacts of energy drinks on the brain and pancreatic tissues of pregnant Wistar rats.

Material and Method: There were three groups of pregnant rats, each having six animals. The low-dose and high-dose groups got 5 and 10 ml/kg body weight of an energy drink orally, respectively. The control group received distilled water. From the 5th to the 19th day of pregnancy, energy drinks are given. Additionally, antioxidant enzymes including glutathione reductase (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured to determine oxidative damage in the brain and pancreatic tissues. histopathological analysis and biochemical markers, DNA degradation, were also examined.

Results: Energy drinks significantly decreased SOD and GSH in the brain and pancreatic tissues of both treated groups compared to the control group (p-*value*<0.05). In addition, both treated groups had significantly lower brain tissue dopamine and acetylcholinesterase levels (p-*value*<0.05). Compared to the control group, the treated groups showed significant increases in MDA levels, serum amylase activity, serum lipase activity, serum glucose levels, and OTM values in brain and pancreatic tissues (p<0.05).

Conclusion: Administration of energy drinks orally to pregnant Wistar rats resulted in evident harm to the brain and pancreatic organs, possibly due to oxidative stress and raised generation of free radicals.

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Key Words: Brain and pancreatic tissues, dopamine, DNA degradation, energy drinks.

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INTRODUCTION

The necessity to be available around the clock, the accelerated pace of life, and ubiquitous haste have led to a rise in the consumption of caffeinated energy drinks (EDs) by some populations[1]. The phrase "energy drink" (ED) describes drinks that are thought to boost cognitive function, lessen weariness, improve physical performance, and enhance personality[2].

Energy drinks are consumed by consumers for a variety of purposes, including partying with friends or enhancing their performance in academic pursuits, driving, staying up late, or sports[3]. The idea that EDs can improve physical strength, speed up reactions, reduce the need for sleep, and keep the body alert with a higher mental focus state has led to an increase in ED usage worldwide[4].

When energy drinks first hit the American market in 1997, they gained recognition on a global scale[5]. Caffeine, taurine, guarana, sugar, salt, and vitamin B6 are typically included in their contents[6].

Energy drinks primarily contain caffeine (1,3,7-trimetilxantine) as their psychoactive ingredient. High amounts are typically found in it, which frequently leads to overindulgence and poisoning symptoms[6–10]. While caffeine has been linked to enhanced cognitive functions including focus and memory, its full range of effects—whether when taken alone or in combination with other substances—remains to be discovered[6]. Orally, caffeine is quickly and completely absorbed, and its half-life of elimination is roughly 4.5 hours[10,11]. Caffeine functions as a psychostimulant in rats and humans[12,13], elevating anxiety and alertness while decreasing weariness. According to pharmacological research, the antagonistic activity of caffeine at the A1 and A2 adenosine receptor subtypes mediates its effects on the central nervous system[14].

The information that is now available indicates that it could be wise for expectant mothers to restrict their coffee intake to three cups per day, with a daily maximum of 300 mg of caffeine, in order to rule out any increased risk of

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spontaneous abortion or stunted fetal development. The gastrointestinal tract's mucosa quickly absorbs caffeine. It quickly passes through the placenta and reaches a concentration in the fetus that is comparable to that in maternal plasma. Low birth weight (LBW), intrauterine growth restriction (IUGR), spontaneous abortion, and premature delivery have all been linked to caffein[15]. According to a report, the wide range of ingredients that make up EDs is more likely to cause negative effects than only caffeine[1].

Energy drinks contain the dietary additives taurine, glucuronolactone, and gluconolactone, which are also common mammalian metabolites and naturally occurring substances in food at much lower concentrations. Unwanted effects on the heart, kidneys, and brain are caused by high chronic energy drink use, which exposes people to higher levels of taurine, gluconolactone, and glucuronolactone on a daily basis than the predicted mean. Adults who regularly consume food additives included in energy drinks and soft drinks are at risk for developing a number of neurological conditions, such as migraines, seizures, endocrine problems, and neuropsychiatric problems[16]. It was reported that downing a cold beverage quickly leads to a larger peak plasma concentration of caffeine than sluggishly sipping a hot beverage, which has more adverse effects even at lower doses[17]. Heart patients, expectant mothers, adolescents, young children, and those who are sensitive to caffeine are at higher risk[18].

On one hand, it has been found that EDs cause a number of adverse consequences, the most common of which are neurological, cardiovascular, and hepatic damage; they do not improve memory[19-21]. Additionally, a number of studies have shown that consuming high-sugar beverages increases the risk of type 2 diabetes[21].

So, the present study examined the levels of oxidative stress indicators in the brain and pancreatic tissues of pregnant Wistar rats. The objective was to evaluate the effects of energy drinks on these tissues. Moreover, a biochemical analysis of amylase, lipase, and glucose. The effects of energy drinks were evaluated using the Comet test to quantify the extent of DNA degradation, as well as measuring insulin levels in the blood serum, dopamine and acetylcholinesterase levels in brain tissues, and examining any histopathological changes.

MATERIALS AND METHODS

Chemicals

The energy drink is available in Egypt's local market, whereas Bio Diagnostics in Cairo, Egypt where the solvents, including saline, came from.

Animals

Eighteen fully developed female Wistar albino rats were selected from the National Cancer Institute, Cairo, Egypt. The rats were kept in a laboratory animal unit situated at the Department of Zoology, Faculty of Science, Cairo University. Throughout the study, their weight varied between 160 -180g. The animals were kept in controlled surroundings with regulated temperature (20–23 °C), humidity (40–50%), and a 12-hour cycle of light and darkness. They might acclimatize to the experimental setup and having access to food and drink for a week.

Mating process

Rats were kept separately for mating purposes; two females were selected and kept in a cage with one male for the entire night. Vaginal smears were obtained from female rats and examined the next morning. A tiny quantity of saline was pushed into the rat's vaginal opening using a pipette. Two drops of the resultant vaginal fluid, which contained cell suspension, were placed on the slide and coated with 0.1% methylene blue. The smears were inspected under a microscope with a 100x magnification after they had dried. The presence of sperm in the examined vaginal smears indicated the location of gestation day zero[22].

Experimental design

Energy drinks are administered for 15 days during pregnancy, starting on the 5th and ending on the 19th day of gestation.

Three groups, each involving six pregnant rats, were established:

Control group: Oral distilled water was administered to the control rats.

Low dose group: The corresponding amount for people, 5 ml/kg body weight of energy drink, was given orally to the low dose group[23].

High dose group: An oral energy drink dosage of 10 milliliters per kilogram of body weight was given to the high dose group, which is an overdose for humans[23].

Specimen collection

The experiment terminated on the twentieth day of pregnancy when all the rats were administered a lethal dose of sodium pentobarbital (at least 150 ml/kg), causing them to become anaesthetized.

A- A heart puncture was used to draw blood, which was then placed in plain tubes to allow for coagulation. The blood was centrifuged for 15 minutes at 4000 rpm and the serum was then stored at -20 °C for further analysis.

B- Using bone forceps to carefully dissect the cranium, brain tissues were extracted[24]. The pancreas was then removed from each rat by making a mid-ventral cut that extended the whole abdominal cavity length to expose the abdominal wall[25]. After being repeatedly cleaned with saline to remove any blood impurities, the pancreas and brain tissues were preserved for additional examination.

Biochemical analysis

A bio-diagnostic kit employing the enzymatic colorimetric approach was employed to measure the

glucose level[26] (CAT. No. GL 13–20), as well as the amylase activity[27] (CAT. No. AY 10 50). In addition, lipase activity was measured using a colorimetric technique with a spectrum kit[28] (CAT. No. 281 001). The insulin level was measured using the ELISA method[29] with a Sigma-Aldrich kit (CAT. No. SE120069).

Oxidative stress markers evaluation

The levels of MDA[30], SOD[30], and GSH[30] were quantified in brain and pancreatic tissues using a colorimetric method with bio-diagnostic kits (CAT. No. MDA 2529), (CAT. No. SOD 2521), and (CAT. No. GSH 2511), respectively.

Dopamine and acetylcholinesterase determination in brain tissue

The ELISA technique from an Abcam kit (CAT. No. SE120069) was employed to quantify the concentration of dopamine. Additionally, the activity of acetylcholinesterase (CAT. NO. MAK119) was determined using a Sigma-Aldrich kit using an enzyme-catalyzed kinetic process.

Comet analysis

Single-cell gel electrophoresis (SCGE), also referred to as the comet assay, is a commonly employed method for determining DNA damage in specific cells DNA damage to cells, such as strand unravels and fragments, was segregated from intact DNA under the influence of an electrophoretic field, resulting in a comet-tail structure visible under a fluorescence microscope. There have been prior explanations of this technique[31-33]. To put it briefly, the slides were examined under a fluorescence microscope after being exposed to 20 µg/mL of ethidium bromide. For instance, a computerized image analysis system captured 100 random comet shapes on each slide. Next, TriTek Comet ScoreTM software (TriTek Corp.) was utilized by the system to assess the images and calculate the properties of the comet. To evaluate the results, standard parameters (tail DNA and tail moment) were developed. The Olive Tail Moment (OTM) is the name given to the tail moment parameter using the following equation (1) which, according to DNA mobility and abundance in the tail, is believed to be the best sign of DNA damage.

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

Histopathological study

Following a biopsy, the pancreatic and brain tissues were promptly preserved in 10% natural buffered formalin. In a day, the fixative was changed. The tissues were then dehydrated using a graded ethanol series. Subsequently, the material underwent xylene cleaning, paraffin wax embedding, 5 µm sectioning, hematoxylin and eosin (H&E) staining, and light microscopy examination by a blinded histopathologist at Cairo University's Faculty of Veterinary Medicine[34,35].

Statistical analysis

Using the one-way analysis of variance (ANOVA) using SPSS, all the data was supplied as means mean ± Standard Deviation comparison between more than two distinct groups. For intergroup comparisons, post hoc analysis was carried out using the Tukey HSD test, and a *P value*<0.05 was deemed significant.

RESULTS

Oxidative stress markers

The oral administration of energy drinks led to a significantly elevated mean level of MDA ($p<0.05$), as shown in (Table 1). Compared to the control group, the treated groups exhibited significantly reduced mean levels of SOD and GSH ($p<0.05$).

Biochemical assays

(Table 2) provides a concise overview of the effects of energy drinks on insulin, glucose, lipase, and amylase. When comparing the low and high-dose treated groups to the control group, there was a statistically significant rise in the average level of serum glucose ($p<0.05$).

Dopamine and acetylcholinesterase levels in brain tissue

(Table 3) provides a summary of the effects of energy drinks on acetylcholinesterase and dopamine levels. The treated groups revealed significantly reduced mean levels of dopamine and acetylcholinesterase in the brain tissues compared to the control group ($p<0.05$).

Comet assay result

The Comet test was used to evaluate DNA damage in the pancreatic and brain tissues (Tables 4,5, Figures 1,2). The energy drink treate groups demonstrated a statistically significant rise in the olive tail moment (OTM) value in both brain and pancreatic tissues ($p<0.05$) compared to the control group.

Histopathological investigations

Pancreatic tissue

(Figure 3) presents the histological analysis results of the pancreatic sections. The results indicated that degeneration, vacuolation, and a significant decrease in cell count led to the reduction in the size of Langerhans' islets.

Brain tissue

(Figures 4-7) show the various regions of the rats' brain that were examined using histological examination. Administering energy drinks to the brain led to various adverse effects, such as neuron atrophy, pyramidal cell apoptosis, nerve tissue necrosis, expansion of empty spaces nearby nerve cells, and misrepresentation of granular cells in the dentate gyrus with minimal neurofibrillary tangles.

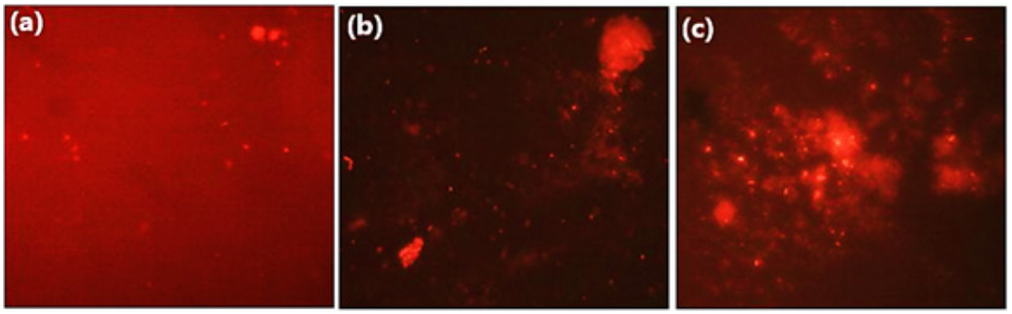

Fig. 1: Effect of energy drinks on DNA degradation in the different studies groups in brain tissue using comet assay. a: Control group, b: low dose group (5ml/kg) and c: high dose group (10ml/kg). b and c show different levels of DNA damage.

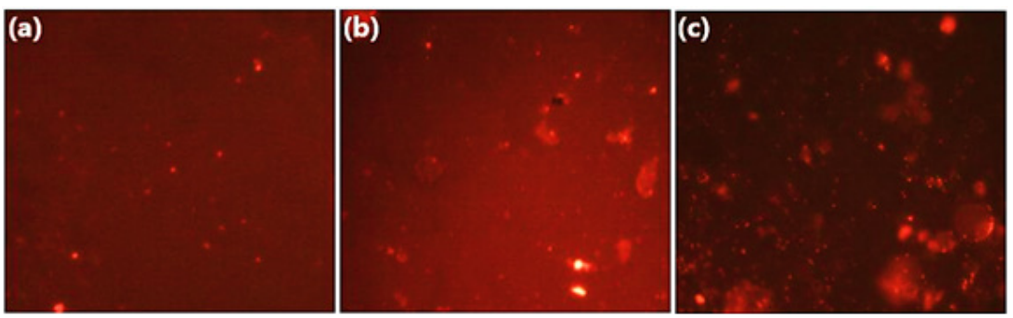

Fig. 2: Effect of energy drinks on DNA degradation of pancreatic tissue in the different study groups using comet assay parameters. a: Control group, b: low dose group (5ml/kg) and c: high dose group (10ml/kg). b and c show different levels of DNA damage.

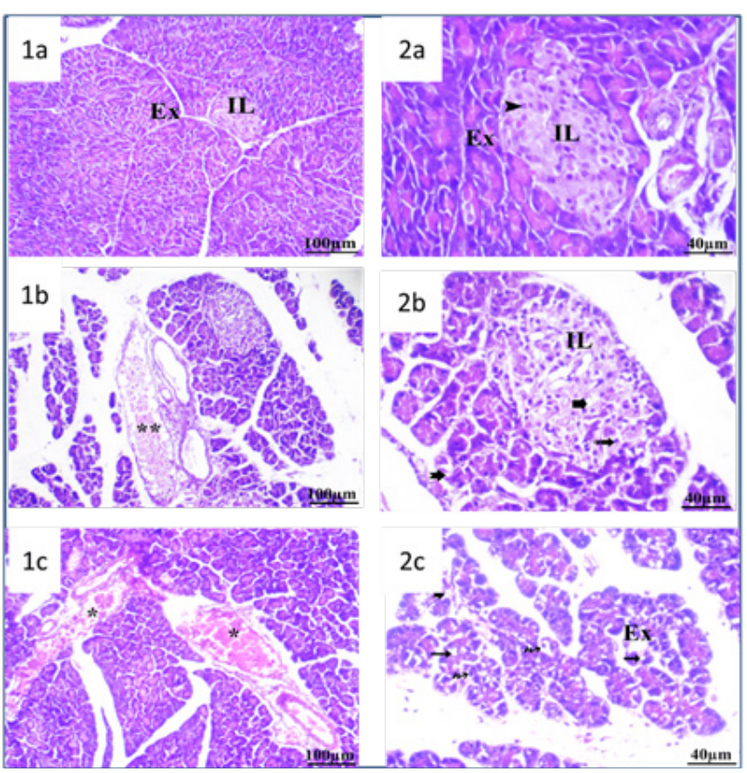

Fig. 3: Photomicrographs of the pancreatic tissue (1a&2a) from the control group show normal histology. The islets of Langerhans (IL) had cords of round or polygonal cells that were weakly stained (arrowhead). Exocrine portion (Ex). (1b&2b) from the low dose group showing dilated and congested blood vessels, exocrine acini (Ex) exhibited acinar damage in the form of cell degeneration (notch arrow), Islets of Langerhans (IL) with vacuolation (bold arrow), pyknosis (dotted arrow), degeneration (notch arrow) of exocrine acini (Ex). (1c&2c) from the high dose group showing most locations had dilated and congested blood arteries (*), many acinar (Ex) and islet cells of the widely separated lobules exhibited cytoplasmic vacuolation (thin arrow), peri-halo cells (wavy arrows) and exocrine acini exhibited acinar (Ex) damage in the form of cell degeneration (notch arrow).

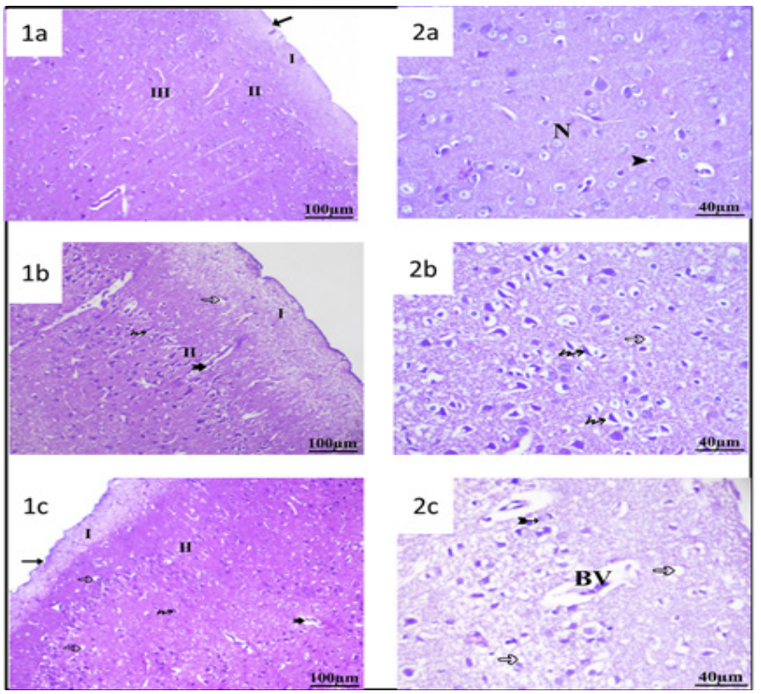

Fig. 4: Photomicrographs of the cerebral cortex (1a&2a) from the control group showing the normal histology. Molecular (I), external granular (II), and external pyramidal (III) are consistently connected by pia matter (arrow). A typical morphological pattern of neurons (N), microglia cells (arrowhead), and a characteristic form of pyramidal cells (N). (1b&2b) from the low dosage group showing a moderate degree of neuropil vacuolation (hollow arrow) and vacuolation of the molecular layer (I), fewer neurons, and darkly coloured shrunken nuclei (wavy arrow). There is moderate blood vessel dilation in the prefrontal cortex layers (BV/notch arrow). The dark, shrinking pericellular haloes of neuronal cell bodies (wavy arrow). (1c&2c): from the high dosage group showing many dilated blood vessels (BV/notch arrow) and dark nuclei with pericellular haloes that are moderately pathologically distinct (wavy arrow). Extensive neuropil vacuolation (hollow arrow) and numerous degraded ghost neurons were observed (bifid arrow).

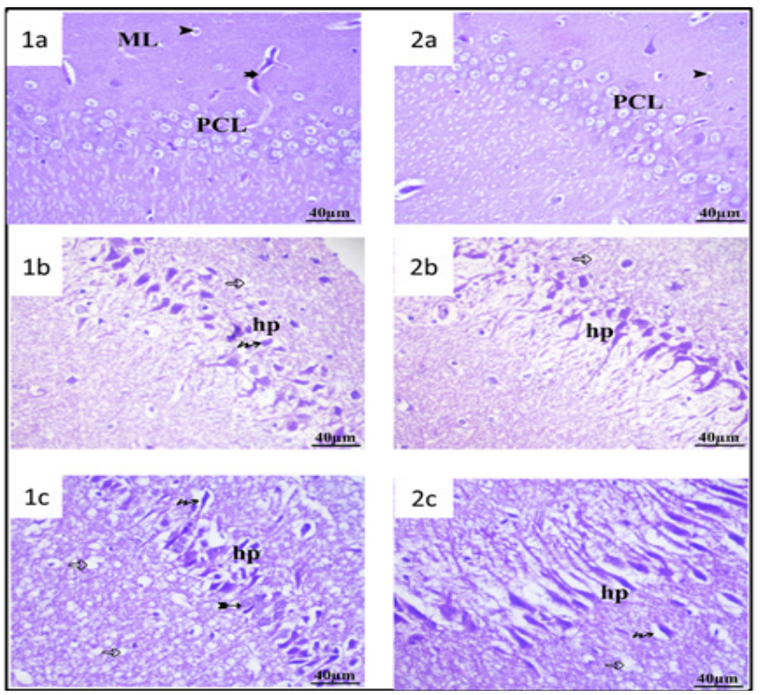

Fig. 5: Photomicrographs of the hippocampus. (1a&2a): from the control group showing normal histology. The pyramidal cell layer (PCL) and molecular layer (ML) of the hippocampus (hp) are in great condition. Glial cells (arrowhead) coexist with regular blood capillaries in the ML (notch arrow). (1b&2b): from the low dosage group showing the pyramidal neuron cell bodies (PCL) are disorganized and loosely packed; they look black and shrunken with pericellular haloes (wavy arrow). (1c&2c): from the high dosage group showing a significantly disorganized PCL; they seem shrunken with pericellular haloes (wavy arrow), while other neurons seemed deteriorated with a ghost-like appearance (bifid arrow).

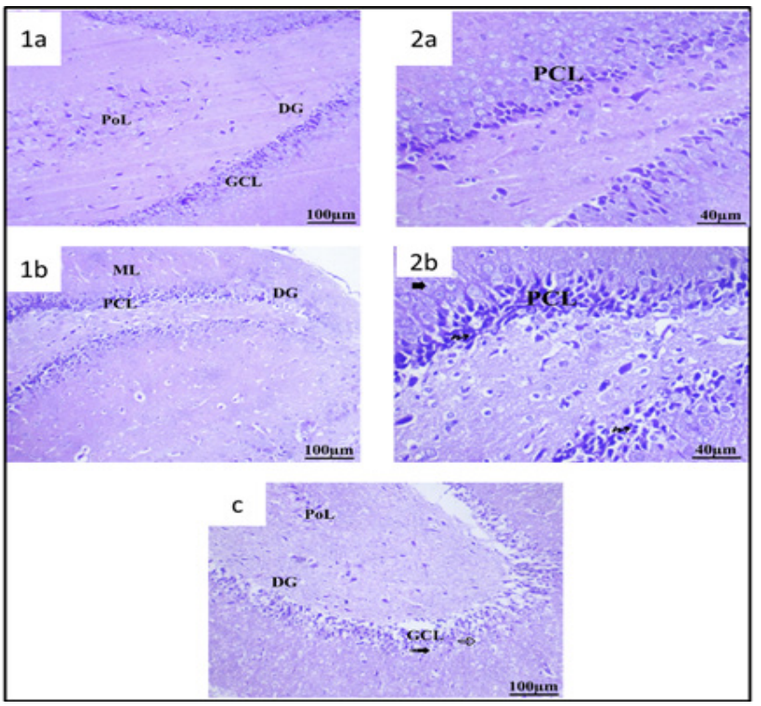

Fig. 6: Photomicrographs of the dentate gyrus. (1a&2a): from the control group showing normal histology. The dentate gyrus (DG) has a delineated granule cell layer (GCL) and polymorphic nuclear layer (PoL). (1b&2b): from the low dosage group showing the granular cell layer (GCL) was disorganized with a degenerated neuron (bold arrow) and dark stained cell (wavy arrow). (1c&2c): from the high dosage group showing the granular cell layer (GCL) was disorderly with vacuolation (hollow arrow) and peri-halo neuron (dotted arrow).

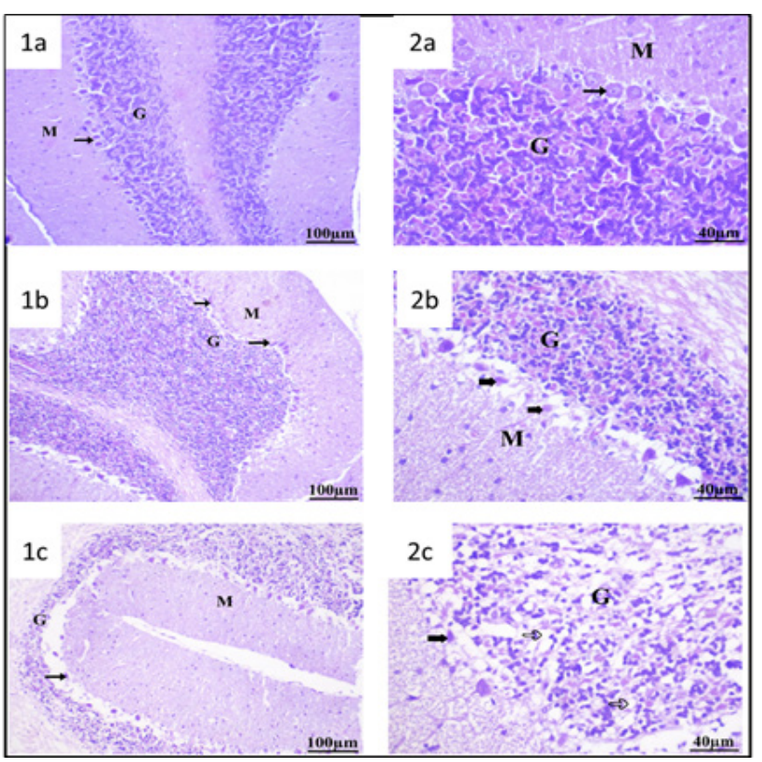

Fig. 7: Photomicrographs of the cerebellum. (1a&2a): from the control group showing normal histology. The cerebellar cortex of rats in the control group contained three layers: outer molecular (M), middle Purkinje cell (thin arrow), and inner granular (G). (1b&2b): from the low dosage group showing that Purkinje cells were observed to have deteriorated into an irregular and deeply stained form (bold arrow). (1c&2c): from the high dosage group showing several Purkinje cell bodies had reduced, uneven forms (bold arrow) and nuclei that were difficult to distinguish. A dilation of the perineural space forms a halo of space around the Purkinje cells (notch arrow).

Table 1: The effect of energy drinks on SOD, GSH, and MDA levels in the brain and pancreatic tissues in the different studied groups.

Parameters / Groups	Pancreatic SOD (U/g.tissue)	Pancreatic GSH (mmol/g.tissue)	Pancreatic MDA (nmol/g.tissue)	Brain SOD (U/g.tissue)	Brain GSH (mmol/ g.tissue)	Brain MDA (nmol/g.tissue)
Control group	6.39±1.53	2.56±0.079	18.41±1.63	14.72±0.44	0.81±0.079	19.36±1.46
Low dose group (5ml/kg)	5.34±0.92 [a]	1.77±0.30	35.81±1.20 [a]	9.95±1.81	0.69±0.070 [a]	20.67±0.30 [a]
High dose group (10ml/kg)	2.59±0.22 [a,b]	0.545±0.09 [a,b]	40.45±1.43 [a,b]	8.33±0.82 [a,b]	0.42±0.055 [a]	24.77±2.76 [a,b]

Six values (mean ± SD) are displayed for each data set. a (p<0.05) in contrast to the control group. In contrast to the low-dose group, b(p<0.05).

Table 2: The effect of energy drinks on glucose, insulin, amylase, and lipase levels in the serum in the different studied groups.

Groups / Parameters	Control group	Low dose group (5ml/kg)	High dose group (10ml/kg)
Glucose (mg/dl)	102.83±11.91	128.67±4.63 [a]	106.3±9.58 [b]
Insulin (µIU/ml)	19.02±0.85	16.68±0.66 [a]	20.21±0.59 [a,b]
HOMA IR	4.81±0.46	5.3 ±0.29	5.21±0.54
Amylase (U/L)	1236.83±77.28	1437.83±64.97 [a]	1445.5±49.91 [a]
Lipase (U/L)	475.00±16.36	599.16±33.31 [a]	607.83±31.01 [a]

Six values (mean ± SD) are displayed for each data set. a (p<0.05) in contrast to the control group. In contrast to the low-dose group, b (p<0.05).

Table 3: The effect of energy drinks on dopamine and acetylcholinesterase levels in the brain tissue in the different studied groups.

Group / Parameters	Control group	Low dose group (5ml/kg)	High dose group (10ml/kg)
Dopamine (mg/dl)	144.7±5.53	122.5±3.68[a]	114.68±7.19 [a]
Acetylcholinesterase (U/L)	15.63±0.71	14.03±0.54 [a]	12.02±0.64 [a,b]

Six values (mean ± SD) are displayed for each data set. a (p<0.05) in contrast to the control group. In contrast to the low-dose group, b (p<0.05).

Table 4: The parameters of the Comet assay for DNA damage in brain tissue across different study groups.

Brain tissue			
Item	Control group	Low dose group (5ml/kg)	High dose group (10ml/kg)
% DNA in tail	6.83±1.42	7.05±1.74	12.15±1.82[a,b]
Tail moment	0.60±0.14	1.07±0.14[a]	0.95±0.12[a]
Olive tail moment (OTM)	1.074±0.291	1.561±0.334[a]	1.922±0.247[a]

Six values (mean ± SD) are displayed for each data set. a (p<0.05) in contrast to the control group. In contrast to the low-dose group, b (p<0.05)

Table 5: The parameters of the Comet assay for DNA damage in pancreatic tissue across different study groups

Pancreatic tissue			
Item	Control group	Low dose group (5ml/kg)	High dose group (10ml/kg)
% DNA in tail	2.45±0.44	5.89±1.82[a]	8.52±2.41[a]
Tail moment	0.44±0.17	0.74±0.19[a]	0.77±0.19[a]
Olive tail moment (OTM)	0.452±0.099	1.516±0.311[a]	1.947±0.300[a,b]

Six values (mean ± SD) are displayed for each data set. a (p<0.05) in contrast to the control group. In contrast to the low-dose group, b (p<0.05)

DISCUSSION

A mother's exposure to toxins during pregnancy may differ from that of a non-pregnant woman at the same dose[36]. As a result, various physiological changes have occurred, such as changes in the pharmacokinetics of toxins, an increase in the volume of distribution of most drugs due to the increased plasma volume and body fat storage, and an increase in the amount of free medicine that can reach target organs during pregnancy due to a decrease in albumin levels and an increase in cardiac output[37].

During pregnancy, significant levels of the female sex steroid hormones progesterone and estrogens are produced, and these amounts rise as the pregnancy goes on. These hormones have vital supporting roles in both preserving pregnancy and ensuring a healthy, full-term delivery. Additionally, they have significant effects on the brain during pregnancy, usually by modifying the effects of circulating peptide hormones on the brain or by interacting with neuropeptide systems in the brain[38].

Insulin, which is secreted by pancreatic beta cells in a manner depending on glucose concentration, regulates glucose homeostasis. Insulin production is insufficient in those with type 1 and type 2 diabetes to keep blood sugar under control. In the former, there is a nearly total loss of beta cell mass, but in the later, there is just a partial shortfall. Pregnancy has been shown to cause rodents' beta cell mass to expand by two to five times[39].

Lipid peroxidation generates MDA, which is detrimental to cellular membranes and interferes with enzyme activity, ion exchange, and cell membrane permeability. The findings agree with previous studies that have demonstrated a significant increase in MDA levels in both the brain and pancreatic tissues after the consumption of energy drinks[40]. The rats exhibited heightened levels of oxidative stress following their intake of energy drinks.

Tissue damage can occur due to an excess of reactive oxygen species (ROS) and reactive nitrogen species (RNS) through many mechanisms. Energy drinks have been found to elevate the levels of superoxide radicals, leading to a notable decrease in the activity of antioxidant enzymes, as seen by the considerable fall in SOD and GSH values. These crucial antioxidant enzymes collaborate with the non-enzymatic antioxidant system to shield cells from free radical-induced oxidative damage[41]. Exposure to caffeine induces cellular pro-oxidant activity, leading to enhanced protein oxidation. Caffeine significantly elevated blood urea nitrogen levels and also induced the activation of xanthine oxidase, hence promoting the synthesis of superoxide anion, H_2O_2, and uric acid through xanthine oxidation. Also, Superoxide dismutases can convert two superoxide molecules into a single molecule of hydrogen peroxide (H_2O_2) and one molecule of water[42]. The interaction between H_2O_2 and O2 resulted in the formation of free radicals[43]. The findings align with previous studies which found that the use of energy drinks led to a significant reduction in the levels of SOD and GSH in both the brain and pancreatic tissues[44]. However, there are conflicting studies on the effect of energy drinks on SOD levels. Studies have demonstrated that energy drinks have a notable impact on increasing levels of brain GSH and SOD[40].

The high carbohydrate content of the energy drinks being tested led to a rise in blood glucose levels, which negatively impacted the entire metabolic process[44]. The reason for this can be attributed to the presence of caffeine, which has been proven to increase hyperinsulinemia through various pathways[45]. These processes encompass increasing cortisol and adrenaline concentrations in the bloodstream, reducing tissue responsiveness, inhibiting glucose utilization, and eventually sustaining elevated blood glucose levels. In addition, the treated groups exhibited significantly elevated mean serum lipase and amylase levels compared to the control group ($p<0.05$). It is hypothesized that this happens by obstructing the flow of lymphatic and venous fluids from the pancreas and surrounding tissues[46]. Free radicals can directly damage cell membranes by peroxidizing lipids that are essential components of the phospholipid structure within the membrane. Enhanced capillary permeability, indicative of the breakdown of endothelial cells, is a prevalent indication of this damage. The release of enzymes at a high level is facilitated by the improved permeability[47]. Capillary leakage is believed to have a substantial impact on this process.

Taking energy drinks dramatically decreased the overall levels of norepinephrine, dopamine, and gamma-aminobutyric acid in several regions of the brain. The observed phenomenon can be explained by the impact of caffeine, which inhibits the adenosine receptors in the brain. This inhibition leads to an increase in the influx of calcium ions into nerve cells and the subsequent release of neurotransmitters stored in vesicles in presynaptic cells through the protein synapsin 1. As a result, the overall quantity of neurotransmitters within cells is reduced[48,49]. The findings agree with previous studies that demonstrate a significant decrease in brain dopamine levels after consuming energy drinks[49,50]. However, there are conflicting data regarding the role of coffee in increasing dopamine levels in brain tissue[51].

Caffeine is an alkaloid belonging to the xanthine (1,3,7-trimethylxanthine) class. The primary therapeutic effect of this psychostimulant is due to its nonselective antagonistic action on the adenosine receptors A1 and A2A. Furthermore, this substance also exhibits inhibitory effects on phosphodiesterase (PDE) and acetylcholinesterase (AChE) enzymes, in addition to releasing intracellular calcium[52]. The human acetylcholinesterase hAChE enzyme was used to study the binding interactions of caffeine to comprehend the AChE inhibitory profile[53]. According to this study, the xanthine ring scaffold was close to the catalytic triad, which is composed of Ser203, Glu334, and His447. The findings align with previous studies that showed a notable decrease in acetylcholinesterase activity in brain tissue after the use of energy drinks[52,54,55].

The energy drinks employed in this investigation contain the artificial sweetener acesulfame potassium. Under normal storage conditions, it stays chemically stable in foods, beverages, and cosmetic formulations.

Acetone, CO2, and ammonium hydrogen sulphate can be produced as a result of significant breakdown caused by extreme pH and temperature conditions, which is also referred to as amido-sulfate, as the end products of the decomposition process. Under acidic conditions (pH 2.5), acetoacetate and acetoacetate N-sulfonic acid are formed as unstable intermediate products of the decomposition process, while under alkaline conditions (pH 3–10.5), acetoacetic acid and acetoacetate N-sulfonic acid can be detected. These degradation products can cause breaks in DNA strands. Additionally, prior studies on the elevated rate of damage at a five-ppm dosage of acesulfame potassium indicated that smaller fracture sizes would lead to swifter electrophoretic movement[56].

In addition, the production of comets demonstrated that the intake of sweets induced a rise in DNA single-strand breaks, with the extent of damage depending on the concentration of the sweeteners. The DNA impairment seen in this study may have arisen from the interactions between sweeteners and their metabolites with DNA, leading to the creation of DNA adducts, double-strand and single-strand breaks in DNA, and cross-links between DNA and proteins[57]. The precise method via which sorbitol, aspartame, saccharin, and acesulfame potassium cause damage to DNA strands is still unknown. Furthermore, there is absence of knowledge concerning these sweeteners and the resulting metabolites that lead to these DNA breaks[58].

The incidence of cytoplasmic vacuolations in the islet cells of the group treated with an energy drink can be assigned to the buildup of degenerative components inside the cytoplasm and the occurrence of fatty degeneration. The vacuolations may be caused by oxidative stress mediated by energy drinks. This stress causes harm to both the cell and organelle membranes, resulting in a rise in their penetrability and a disruption of energy-dependent Na+ and K+ ion pumps. Consequently, the deposit of Na+ occurs within the cells when water enters, leading to cellular enlargement and the formation of vacuoles[59].

The findings align with preceding findings that have demonstrated a substantial boost in blood glucose levels after consuming energy drinks[60,61]. Moreover, most exocrine acini exhibited indications of acinar damage, including cellular degradation and infiltration of a small number of mononuclear cells into enlarged and obstructed blood channels. The pancreatic tissue exhibited several hemorrhagic regions interspersed throughout the exocrine acini.

The lobules contained many islet and acinar cells, which exhibited vacuolation both around the cell membrane and within the cells themselves. Enlarged, obstructed blood arteries with leakage of red blood cells into the spaces amongst the acini. These findings align with the consequences of previous investigators[62], who connected these alterations to abnormalities in the microcirculation caused by an excessive amount of nitric oxide generated due to the oxidative stress condition. As a result, the blood vessel walls become more fragile and are prone to rupturing within the pancreatic tissue, leading to endothelial dysfunction, including the detachment of endothelial cells from the blood vessel walls[63].

The damage in brain tissue may be attributed to caffeine[48,49]. The study revealed that the intake of energy drinks had a substantial effect on reducing the overall number of neural connectors in all areas of the brain. This is primarily due to the caffeine in the energy drink, which induced the blockage of adenosine receptors. As a result, there is an increase in the influx of calcium into nerve cells and a decrease in the overall level of the neurotransmitter being investigated.

The present investigation observed blood vessels exhibiting perivascular oedema and haemorrhaging, together with congested blood vessels within the lamellar area of the cortex. The finding supported the conclusions of previous researchers, who proposed that disturbances in neuronal glucose transport and metabolism during episodes of high and low blood sugar levels could lead to vascular injury.

The presence of pericellular gaps around the neurons can be attributed to the contraction of the cells and the retraction of their processes due to cytoskeletal damage. The observed neuronal necrosis is in line with what is typically seen in the initial stages of ischemia, hypoxic/ischemic hypoglycemia, and excitotoxic circumstances, and it indicates the death of neurons.

The observed cytoplasmic vacuolation in nerve cells may be attributed to injury to the cell membrane and the membranes of other organelles, along with the occurrence of lipid peroxidation. The clear consequence of such damage is a rise in sodium permeability that exceeds the pump's capacity to remove the sodium. The cell undergoes swelling due to an elevation in water content caused by the accumulation of salt[64].

CONCLUSION

The primary purpose of this study was to assess the effect of energy drinks on the brain and pancreatic tissues of pregnant Wistar rats. The study's findings, corroborated by increased MDA and decreased SOD and GSH levels, indicate that everyday consumption of energy drinks during pregnancy leads to brain and pancreatic impairment, along with other adverse consequences. In addition, there is an increase in the concentrations of glucose, lipase, and amylase. The detrimental effects are additionally demonstrated by the histology observations of the examined tissues. It is recommended to conduct further research to develop a withdrawal approach that may effectively eliminate the adverse effects of using energy drinks.

CONFLICT OF INTERESTS

There are no conflicts of interst.

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الملخص العربى

التغبيرات البيوكيميائية والسمية والنسيجية لمشروبات الطاقة علي انسجة المخ والبنكرياس لجرذان الويستار الحوامل

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مقدمة: علي الصعيد العالمي، ما فتئ استهلاك المشروبات من الطاقة يتزايد باستمرار . ويمكن أن يؤدي التركيب الكيميائي لمشروبات الطاقة إلى آثار ضارة مختلفة، مثل الأعراض العصبية مثل الهزات والرعشات، والاضطرابات، وأعراض القلب مثل النزعات القلبية أو التسارع في القلب، والأعراض المعوية الحادة أحيان ا . وكان الهدف من هذه الدراسة هو تقييم الآثار الضارة المحتملة لمشروبات الطاقة على الدماغ والأنسجة البنكارياسية لجرذان ويستار الحامل.
الهدف من البحث: يهدف هذا البحث الي دراسة التأثيرات الضاره لمشروبات الطاقة علي انسجة المخ والبنكرياس جرذان الويستار الحوامل.
مواد وطرق البحث: كانت هناك ثلاث مجموعات من الجرذان الحامل، كل منها لديها ستة حيوانات . وحصلت مجموعتا الجرعة المنخفضة والجرعات العالية على مشروب الطاقة عن طريق الفم بكمية ٥ و ١٠ ملليلتر / كغ من وزن الجسم . وتلقت مجموعة الضابطه مياه مقطرة . من اليوم الخامس وحتى اليوم التاسع عشر من الحمل . وبالإضافة إلى ذلك، تم قياس الانزيمات المضادة للأكسدة، بما في ذلك المالونديالدهيد و ديسموتاز فائق أكسيد والجلوتاثيون المختزل، لتحديد الضرر المؤكسد في المخ وأنسجة البنكرياس . وتم أيض ا فحص التغيرات في انسجة المخ والبنكرياس ، المؤشرات الكيميائية الحيوية، وتحلل الحمض النووي.
النتائج: تسبب تناول مشروبا ت الطاقة لانخفاض في كمية الديسموتاز والجلوتاثيون المختزل في انسجة المخ والبنكرياس في كلا المجموعات مقارنة بالمجموعة الضابطه بالإضافة الي انخفاض نسبة الدوبامين والاستيل كولين استريز في انسجة المخ في كلا المجموعات المعالجة . المعالجه وعند المقارنة بالمجموعة الضابطه تبين انه في كلا المجموعات المعالجة حدث زياده لنسبة المالونديالدهيد في نسيج كلا من المخ والبنكرياس وزيادة أيضا الاميليز والليبيز والسكر في الدم وكذلك ارتفاع قيمة فحص المذنب الدالة علي معدل تضرر الحمض النووي في نسيج كلا من المخ و البنكرياس.
خلاصة البحث: ونتج عن اعطاء مشروبات الطاقة لجرذان ويستار الحوامل إلحاق ضرر واضح بانسجة المخ والبنكرياس، ربما بسبب الإجهاد المؤكسد وتوليد الجذور الحرة.