Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 1379-1392.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

IMPACT OF ENERGY DRINKS ON KIDNEY TISSUE AND OXIDATIVE STRESS IN FEMALE WISTAR RATS AND THEIR NEONATES

Heba Ali Abd El-Rahman[1], Aya Ragab Abd El-Ghany[2], Mohamed A. El Desouky[2], Demiana H. Hanna[2*]

[1]*Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt*
[2]*Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt*

This study investigated the potential health risks associated with energy drink (EDs) consumption in nursing Wistar rats. Nursing rats were divided into three groups: a control group receiving distilled water, a low-dose EDs group, and a high dose EDs group. Biomarkers of oxidative stress, renal function, and DNA damage were evaluated.

Results demonstrated a significant increase in oxidative stress, as evidenced by elevated malondialdehyde levels and disrupt antioxidant activity, renal function parameters were significantly elevated in EDs-exposed groups, indicating kidney impairment. Histopathological examination revealed renal damage (shrinkage and fracturing in glomeruli -congestion) in EDs exposed rats. Additionally, DNA damage was significantly increased in both mothers and offspring as OTM statistically significant increased.

These findings underscore the potential adverse effects of EDs consumption on maternal and neonatal health. These results emphasize the need for further research and public health interventions to inform consumers about the potential risks associated with EDs consumption during breastfeeding

Keywords: *Energy drinks, oxidative stress, kidney function, pregnancy, lactation, DNA damage*

INTRODUCTION

Energy drinks have become increasingly common among individuals of all ages during physical activity, attributed to a rise in their consumption rate; these beverages have gained prominence in both Arab and worldwide markets[1].

Energy drinks (EDs) are caffeinated, non-alcoholic beverages containing psychotropic substances such as glucuronolactone, amino acids, and botanical extracts like ginseng and guarana. The consumption of EDs may result in detrimental short- and long-term health effects, particularly among the youth[2].

Energy drinks (Red bull) contain caffeine, taurine, sucrose, glucose, citric acid, magnesium carbonate, sodium citrate, inositol, niacin amide, vitamin B12, pyridoxine HCL and artificial flavors[3]. EDs have been associated with hypertension, significant cardiovascular incidents, renal disease, metabolic complications, sleep disturbances, and seizures furthermore high sugar content results in obesity and diabetes have all been caused by EDs[4]. Consumption of caffeinated EDs may induce Hepatitis and pancreatitis[5], nephrotoxicity[6] and hematological problems[7]. It may provoke oxidative stress in multiple organs, diminish antioxidant defenses, increase the production of reactive oxygen species (ROS), damage proteins, lipids, and nucleic acids, and even lead to cell death[8].

Taurine (2-aminoethanesulfonic acid), a sulfonated β-amino acid predominantly synthesized in the liver from cysteine or acquired through dietary sources such as meat and dairy, as well as energy drinks, may halt or reverse cognitive, memory, and learning impairments induced by neurological toxins. Significant concentrations of taurine are present in the heart, liver, and central nervous system, including the brain stem and hippocampus, which play crucial roles in

Received : 25/8/2024 & Accepted : 17/10/2024

*Corresponding author: Demiana H. Hanna, E-mail: dhelmy@sci.cu.edu.eg

osmoregulation, membrane stabilization, neuroprotection, neuromodulation, and the regulation of cellular calcium levels[9]

Caffeine, one of the most extensively ingested alkaloids globally, is present in energy drinks and is rapidly absorbed by the human digestive system, attaining peak levels 45 minutes' post-consumption[10]. With varied degrees of effectiveness, numerous research has been done on the connection between female fertility and caffeine. Most research has been retrospective in nature, examining the relationship between a woman's stage of pregnancy and her caffeine use. They have either shown no fertility at all or lowered fertility[11]. Caffeine or other ingredients in certain energy drinks may have an impact on fertility, while the precise mechanisms remain unclear. Changes in estradiol and other hormones have also been associated to caffeine[12]

Pregnant women should restrict their caffeine use, as the half-life of caffeine metabolism is considerably prolonged during pregnancy. Elevated doses may adversely affect fetal growth, lead to congenital anomalies, or increase the likelihood of miscarriage[13]**.** The safe consumption level of caffeine remains undetermined in scientific studies. Women who consumed less caffeine during pregnancy may have experienced smaller gestational ages, lower birth weights, or stillbirths, while those who ingested larger amounts of caffeine may face an increased risk of gestational problems[14]**.**

Caffeine is rapidly absorbed by the gastrointestinal tract upon consumption. The blood reaches its peak level within 30 to 120 minutes. Caffeine is transported to the body's tissues via the bloodstream. Caffeine traverses the placenta to access the fetus, amniotic fluid, and breast milk after penetrating the blood-brain barrier. A breastfed infant may experience negative effects if the nursing mother ingests excessive caffeine. Excessive caffeine consumption in children can lead to muscle tremors and increased tension. Furthermore, when breastfeeding mothers consume high levels of caffeine, their infants may exhibit anxiety, insomnia, convulsions, nausea, and irritability[15].

This study sought to examine the impact of EDs on the renal tissues of mother rats following breastfeeding and pregnancy, as well as on the renal tissues of their offspring. Consequently, measurements were conducted on oxidative markers including glutathione reductase (GSH), superoxide dismutase, and malondialdehyde. Furthermore, the levels of urea, uric acid, and creatinine were assessed. Furthermore, all examined groups exhibited histopathological changes and DNA degradation in their renal tissues.

MATERIAL AND METHODS

Chemicals

Energy drinks (Red pull) , all chemicals and reagents were purchased from Bio Diagnostic Company, Dokki, Giza, Egypt.

Eighteen female Wistar albino rats weighing 200 and 220 grams were raised in the Department of Zoology's laboratory animal unit at the University of Cairo Faculty of Science. The animals were kept in 65x25x15 cm polyethylene cages with sawdust floors, 12 hours of light and 12 hours of darkness, 20–23 °C temperature, and 40–50 % humidity. All animals had access to food and water and were given a week to acclimate to the experimental conditions. The care of all experimental rats was authorized by the Institutional Animal Care and Use Committee (animal ethics acceptance number: CU-I-F-48-22).

The animals were split up randomly into three groups, with six animals in each group:

The first group (control group), in which distilled water was given to the pregnant rat via a gavage tube. The second group (low dose group) in which the pregnant rat obtained 5mL/kg body weight of energy drinks[16]. The third group (high dose group) in which the pregnant rat obtained 10 mL/kg body weight of energy drinks. The dosage was administrated daily from the fifth day of pregnancy to the twenty-first day of post- partum.

Blood Sample and tissue preparation

At 22 days after giving birth, the mother rats and their newborns were humanely killed, and their tissues were collected. Tissue samples from the kidneys of mothers and neonates were rinsed with saline solution and stored at a

temperature of -20°C immediately after euthanasia. These tissue samples were used for comet assay and to measure oxidative stress markers such as MDA, GSH, and SOD. The tissues were homogenized in PBS and then centrifuged at 4000 rpm to create a tissue homogenate. Whole blood was collected in plain tubes and allowed to coagulate for 10 minutes, the serum samples were obtained by centrifuging the clotted blood at 4000 rpm For 5 minutes The resulting serum was then stored at -20 °C to analyze biochemical biomarkers such as creatinine, urea, and uric acid.

Measurement of oxidative stress markers

The kidney tissues homogenate were utilized to measure the activities of MDA[17], SOD[18], and GSH[19] using a colorimetric method from bio diagnostic kits (CAT. No. MDA 25 29), (CAT. No. SOD 25 21), and (CAT. No. GSH 25 11), respectively.

Reagent for measurement of parameter

MDA

MDA standard (10 nmol / mL) – buffer chromogen (Thiobarbituric acid, detergent, Stabilizer 25 mmol / L).

Thiobarbituric acid (TBA) reacts with (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm.

SOD

Phosphate Buffer pH 8.5 (50 mM/L) - Nitroblue tetrazolium (NBT) (1 mM/L) – NADH (1 mM/L) Phenazine methosulphate (PMS) (0.1 mM/L).

The absorbance recorded at 560 nm for 5 min at 25 °C, the SOD activity was measured on its ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitrobluetetrazolium dye

GSH

Trichloroacetic acid (TCA) (500 mmol / L) – Buffer (100 mmol / L) – DTNB (1.0 mmol / L).

GSH activity was estimated by reduction of DNTB with GSH to produce a yellow compound, the reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

Biochemical analysis

The levels of creatinine[20], urea[21], and uric acid[22] were measured using serum samples, utilizing bio diagnostic kits (CAT. No. CR 12 50), (CAT. No. UR 21 10) and (CAT. No. UA 21 20), respectively. Creatinine level was determined using colorimetric method, the urea was determined by using Urease-Berthelot Method and uric acid was determined using the Enzymatic Colorimetric Method.

Comet assay

The comet assay, called single-cell gel electrophoresis (SCGE), is frequently employed to detect DNA damage in particular cell types. An electrophoretic field causes damaged cellular DNA to segregate from unharmed DNA, forming a structure that appears as a comet's tail under a fluorescence microscope. This procedure has already been described in[23,24]. As follow: firstly, the frosted slides were prepared by covering the slides with 100 ml of agarose (low melting point, 1%) in PBS at 37˚C and the agarose was frozen, the tissues were centrifuged (2000 rpm for 5 min), and re-immersed in ice-cold PBS (step1). Furthermore, about 10 ml of this prepared tissue suspension was added to low melting point agarose (100 ml), and rapidly located on the frosted slide that was previously covered with low melting point agarose (step 2). Subsequently, these prepared agarose slides were incubated at 4˚C for 2 h in cold lysis buffer, resulting in separation of protein-depleted nuclei containing supercoiled DNA (step 3). Next, the control and treated samples were dipped in high alkaline solution for disruption the hydrogen bonding between the DNA strands and also conversion alkali-labile lesions to DNA strand breaks (step 4). Then the slides were placed on horizontal gel electrophoresis with an electrophoresis buffer for 20 min (25 V, 300 mA, and pH 13.0) for construction of single-cell comets (step 5). Following, these slides were immersed in neutralization buffer (pH 7.5) three times for elimination of the extra alkali and detergents to ensure effective staining (step 6). Lastly, an ethidium bromide stain (20 μg/ ml) was used for staining the slides and then observed with a fluorescence microscopy (step 7), such that every 100 images of random comets shape for each slide were captured using a computerized

image analysis system which analyzed these images and estimated the comet parameters (step 8), using TriTek Comet Score™ software (TriTek Corp., Sumerduck, VA, USA). Two metrics frequently used to evaluate the results are tail moment and tail DNA. Applying the subsequent formula[25], the Olive Tail Moment—a tail moment parameter that accounts for DNA mobility and abundance in the tail—is believed to be the most accurate technique to assess DNA damage.
Tail moment × tail /100 is OTM.

Histopathological examination

The materials were preserved for 48 hours at room temperature in 10% neutral buffered formalin[26]. After being cleaned with xylene, the tissues were infiltrated in three changes of melted paraffin at 60 °C for four hours, dehydrated in increasing grades of alcohol (70%, 80%, 90%, 95%, and absolute alcohol) and embedded in newly melted paraffin using a manual tissue schedule. The blocks of paraffin were sealed and refrigerated. Sections were thinned to five microns using a rotary microtome, then placed on glass slides, dried at 37 °C, and stored carefully for staining with hematoxylin and eosin as follow before staining the sections for five minutes with hematoxylin, the sections were rinsed under running tap water for approximately three minutes to allow for bluing, and the stain was separated in 1% acid alcohol for two to three seconds. The slides underwent three minutes of Eosin counterstaining, followed by a water rinse and a one-minute dehydration process using progressively higher alcohol grades (50, 70, 90%, and 100%). After that, the slide parts were quickly cleaned with xylene and allowed to dry for sixty seconds at 80°C in the oven and then covered with microscopic cover glass (22 mm X 50mm) with the aid of DPX (Distrene Plasticizer Xylene) mounting before being examined under a bright field light microscope[27,28].

Statistical analysis

An analysis of the measurements was conducted using SPSS version 29. Data from multiple groups were compared using analysis of variance one- way ANOVA, and then followed by compared between two groups using the Tukey's post hoc test. Every data set was reported as means ± SD, with statistical significance defined as $P < 0.05$.

RESULT AND DISCUSSION

Result

Oxidative stress markers:

According to **Table 1**, the consumption of energy drinks led to a statistically significant rise in MDA levels in kidney tissues of mother rats after gestation and lactation period in both the low-dose and high-dose groups compared to the control group ($p < 0.05$).

The present investigation corroborated earlier findings energy drinks treated groups have increased MDA level compared to control group, this discovery might result from renal impairment, oxidative stress induction, a decline in the kidney's cellular defense mechanism, and an increase in lipid peroxidation levels that raise ROS production and oxidative stress[29].

Energy drinks cause oxidative stress due to their high sugar index, which prolongs hyperglycemia and produces an excessive amount of peroxide free radicals. producing interstitial inflammation, increased proteinuria, and histological changes, which are indicators of oxidative damage[30].

However, when compared to the control group in the mother rats, the low and high-dosage groups' SOD and GSH levels were lower ($p < 0.05$).

This may be similarity explained by the result of consuming energy drinks, which decreased the levels of antioxidant enzymes like SOD and GPX and elevated oxidative stress, These enzymes are important antioxidants that prevent oxidative damage from free radicals in cells by collaborating with the non-enzymatic antioxidant system, Antioxidant enzymes are the first line of defense against cell damage resulting from oxidative stress, By changing the extremely reactive superoxide anion into hydrogen peroxide, which is then broken down into water by GPX and CAT, SOD plays a significant role in neutralizing the anion[31].

Thus, it was considered that taurine and caffeine together can generate oxidative stress, which could account for the increased levels of lipid peroxidation and lower levels of antioxidants.

On the other hand, Memudu[10] claimed that there was increase in SOD level when rats exposed to energy drinks, this might be due to energy drinks raise SOD levels because they produce high amounts of superoxide radicals in tissue during metabolism, which results in the production of hydrogen peroxide and oxygen through catalytic reactions. Hydrogen peroxide deposits in kidney tissue have a toxic effect that prevents the kidney from functioning normally, increasing oxidative stress and ultimately leading to kidney tissue impairment.

The rate at which caffeine is metabolized starts to decline during the initial trimester of pregnancy, and this decline persists until delivery. Furthermore, the blood-placental barrier is ineffective in preventing the transfer of caffeine from the mother to the fetus due to caffeine's lipophilic properties. Additionally, the fetus lacks the necessary enzymes to metabolize caffeine. Consequently, caffeine consumed by women passes directly to the fetus without undergoing any metabolic processes. Historically, it has been suggested that caffeine and its metabolites may induce vasoconstriction in the placenta[14].

Human breast milk is an innate and superior nourishment for babies. It necessitates the ideal combination of nutrients to fulfill their early-life nutritional requirements and offers related immunological and psychological advantages. Breastfeeding is acknowledged to decrease child mortality and provides long-term health advantages that last throughout adulthood[15].

Table (1) shows the number of energy drinks that newborns in the high-dose and low-dose groups drank in comparison to the control group. This causes elevation in MDA levels,

This finding similarity explained by Al-Basher, who stated that there was significant increase in MDA level in mice offspring this may be due to increase in lipid peroxidation level led to elevated levels of reactive oxygen species (ROS) and increase in oxidative stress[8]. When compared to the control group, the SOD and GSH levels in the neonates were higher in the low-dose and high-dose groups ($p < 0.05$).

Table 1: Effect of energy drinks intake on oxidative stress markers in kidney tissues of mother rats and neonates.

	Mather rats tissues		
Parameter	**MDA (nmol/g tissue)**	**SOD (U/g tissue)**	**GSH (mg/g tissue)**
Control	184.53 ± 2.22	473.21 ± 5.67	20.75 ± 1.03
Low-dose (5mL/kg)	218.11 ± 1.60[a]	431.88 ± 7.61[a]	16.15 ± 1.12[a]
High-dose (10mL/kg)	240.79 ± 8.43[ab]	367.08 ±10.85[ab]	10.40 ± 0.84[ab]
Parameter	**Neonatal tissue**		
Control	16.70 ± 1.37	23. 81 ± 1.83	9.15 ± 0.44
Low-dose (5mL/kg)	33.81 ± 4.61[a]	40.46 ± 1.11[a]	14.51 ± 0.41[a]
High-dose (10mL/kg)	58.65 ± 2.41[ab]	69.24 ± 0.41[ab]	19.34 ± 0.44[ab]

Note: data are presented as mean ± SD (n=6). Statistically significant ($p < 0.05$) differences are shown by lowercase letters.

Abbreviations: MDA, malondialdehyde, SOD, superoxide dismutase, GSH, glutathione reductase.

[a] ($p < 0.05$) concerning the control group. [b] ($p < 0.05$) concerning the low-dose group.

This could be because the newborns' immature defense systems and low antioxidative activity are caused by their inefficient natural antioxidant system[32]. According to Frazier[33] The process of renal maturation in rats is unique and usually limited to the period from five days before birth to about fourteen days after birth. Nephrogenesis continues postnatally up to 11 postnatal days and tubule differentiation occur up to 21 postnatal days, and vasculogenesis, or the maturation of blood vessels, occurs up to 19 postnatal days.

Because of their incapacity to activate antioxidant defenses, with increased production of free radicals, and their insufficient antioxidant protection, newborns are especially vulnerable to oxidative stress and damage, the generation of reactive species was out of balance with the biological system's capacity to rapidly detoxify the reactive intermediates or undo the damage that they had caused[34].

Biochemical analysis

Table 2 lists the mother rats' energy drinks during the lactation and pregnancy phases. The levels of creatinine and urea in the low and high-dose groups increased but non-statistically significantly compared to the control group.

This study was agreement with our study as non -significant difference in creatinine and urea level when rats exposed to energy drinks, this may be due to the short duration of red pull intake[35]. According to Muxidinovna, their levels increase because urea and creatinine are byproducts of protein breakdown and build up in the blood when the kidneys are affected[36].

In contrast, according to Akande[37], urea and creatinine levels did not correlate with caffeine consumption.

Although the level of uric acid in mother rats was statistically significant in the low and high-dose groups ($p < 0.05$) when compared to the control group, this finding was similarity explained by Khalaf , who state that there was increase in blood uric acid level when 50 humans who drank energy drinks, might be explained by the consumption of energy drinks, as taurine, a component of energy drinks, is known to transport calcium, potassium, sodium, and water to the cell. It was found that an increase in body salts, particularly sodium salt, might cause an increase in uric acid levels when they exceed acceptable limits, depending on how long energy drinks are drunk[38]. And by Alansari[39], who stated that consumption of caffeine led to raise BUN levels, which activated xanthine oxidase and accelerated xanthine's conversion to uric acid.

The consumption of energy drinks by neonates was compared to the control group in **Table (2).** The findings revealed that increased the level of uric acid level but, non-statistically significant difference and a statistically significant increase in urea and creatinine levels ($p < 0.05$).

Table 2: Effect of energy drinks intake on biochemical parameters in mother rats and neonates.

	Mather rats tissues		
Parameter	**Creatinine (mg/dl)**	**Urea (mg/dl)**	**Uric acid (mg/dl)**
Control	0.48 ± 0.03	9.75 ± 0.83	3.17 ± 0.51
Low-dose (5mL/kg)	0.49 ± 0.06	10.25 ± 0.79	4.94 ± 0.54[a]
High-dose (10mL/kg)	0.50 ± 0.09	11.03 ± 1.18	5.08 ± 0.57[a]
Parameter	**Neonatal tissue**		
Control	0.32 ± 0.02	9.62 ± 0.93	2.63 ± 0.44
Low-dose (5mL/kg)	0.48 ± 0.04[a]	12.45 ± 0.93[a]	2.85 ± 0.55
High-dose (10mL/kg)	0.55 ± 0.05[ab]	15.38 ± 1.66[ab]	3.23 ± 0.52

Note: data are presented as mean ± SD (n=6). Statistically significant ($p < 0.05$) differences are shown by lowercase letters.

[a] ($p < 0.05$) concerning the control group. [b] ($p < 0.05$) concerning low dose group.

This is similarity explained by Alansari[39] who stated the level of creatinine, urea and uric acid are statistically significant increase, that this could be due to the kidney tissue's excretory function disruption. It was also shown that caffeine affected kidney function by inhibiting A2A adenosine receptors, which increased the expansion of inflammatory reactions in interstitial spaces, enhanced proteinuria, and altered renal histology and physiology. Urea is produced during protein metabolism, whereas creatinine is produced by the muscle's breakdown of creatinine phosphate. Fareed[39] states that rat offspring exposed to caffeine it causes a statistically significant increase in creatinine and urea level, the kidney must eliminate urea and creatinine from the bloodstream. As a result, it is widely recognized that elevated serum or plasma creatinine and urea levels indicate a high degree of renal damage. And It has been suggested that taurine and renal failure are linked[40]. Gheith stated that elevated serum creatinine and urea level was interpreted on the basis of caffeine content in energy drinks[1]. A deficient antioxidant system and elevated MDA levels also contribute to renal damage and accelerate the loss of renal function.

This result was confirmed by histopathological examination of the kidneys of mother rats. As seen in **Fig. (1)**, the renal cortex of the control group was densely packed with urine space, glomerular capillaries, Bowman's capsules, and renal corpuscles. At the proximal convoluted tubules' base is a small lumen ringed by cubical cells with spherical nuclei. Simple cubical cells with sphere nuclei in the center or at the apex of the tubule walls surrounded the massive lumen of the distal convoluted tubules.

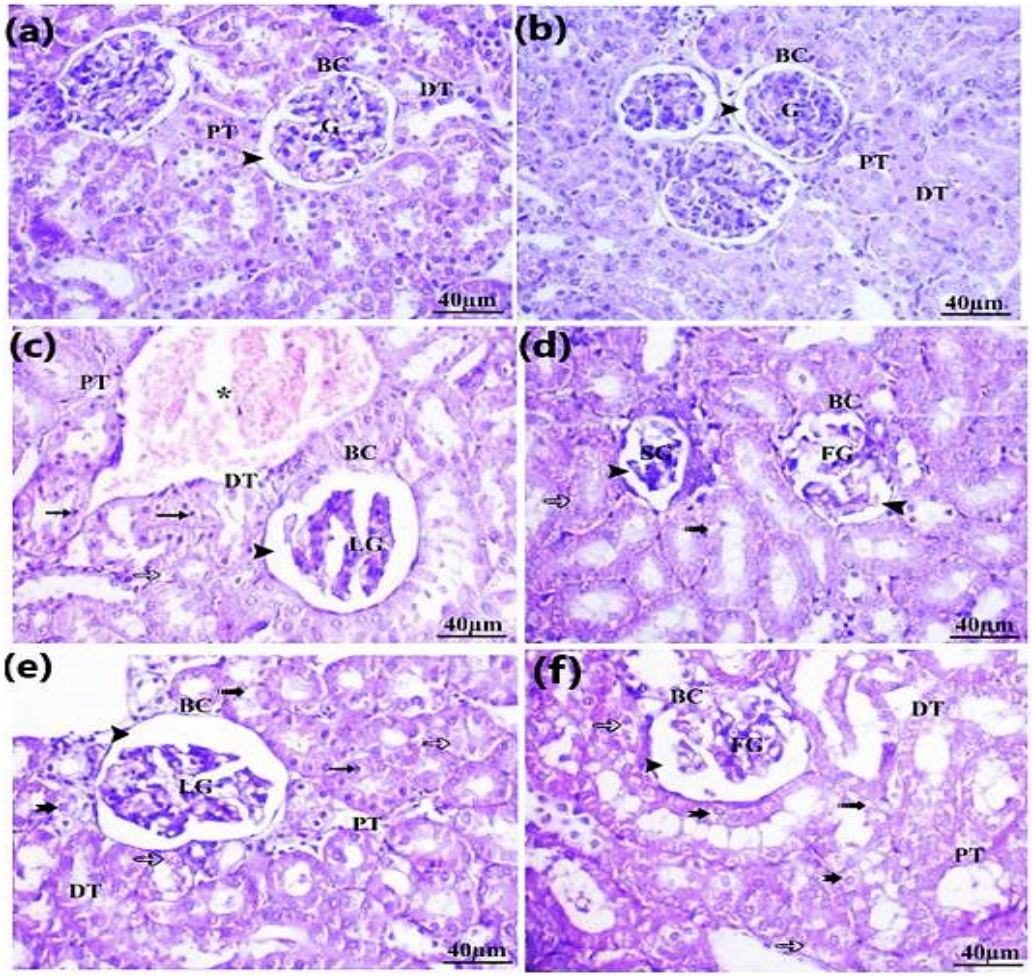

Fig. 1: Photomicrographs of mother rats kidney H&E stain, G: glomerular capillaries, BC: Bowman's capsule, arrowhead: urine space, PT: Proximal convoluted tubules, DT: distal convoluted tubules. a, b: control group. c, d: low-dose group. e,f: high-dose group.

Certain glomeruli in the low-dose group showed signs of shrinkage, fracturing, and lobulation with a broad capsular gap. Complete or partial damage to cell nuclei in the proximal and distal convoluted tubules suggested disintegration and cytoplasmic vacuoles. It has also been noted that the nucleus compacts. There was a fair amount of congestion. Cellular remnants within tubular cells were discovered.

Under light microscopy, H&E-stained renal cortical sections in the high-dose group revealed damage. Some tubules have enlarged lumens and a thin epithelial wall lining them. There were vesicles and vacuolated cytoplasm in several tubular cells. Capillary congestion was experienced. Cell nuclei were injured in both the proximal and distal convoluted tubules, suggesting disintegration and pyknotic dark dead nuclei. The endothelium lining the convoluted tubules had been covered by hydropic degeneration. Within Bowman's capsule, most glomeruli were constricted and had a large capsular gap. There were not many fractured and lobulated.

Current study findings indicate that the histopathological changes observed can be attributed to the high levels of caffeine, sugar, and other substances commonly found in energy drinks. These substances have been shown to increase blood pressure and blood flow. This can lead to renal tubular stress, resulting in dilation of the tubules' lumens and thinning of the walls of the epithelial cells due to increased pressure and alterations in cellular homeostasis. Tubular cells with vacuolated cytoplasm show signs of cellular stress and damage. The chemicals in energy drinks cause oxidative stress and toxins, which could explain this. Cells can become stressed and stop working properly, which causes them to make vacuoles to help them battle the damage. Hydropic degeneration means that cells are getting bigger because of too much water. Biological response to damage that happens when ion pumps in the cell membrane stop working, letting water into the cell.

Khayyat[6] reported that Kidney tissue may be impaired if it is exposed to an excessive amount of the components in energy drinks. This is acceptable because, during excretion and elimination, the renal tubules come into contact with toxic substances. Also EDs may reduce the glomerulus's inner epithelial layer and increased Bowman's space[10]**.** Moreover**,** consuming energy drinks can lead to severe inflammation of the interstitial tissues, a reduction in the size of some renal corpuscles with a narrowing of the urinary space, a markedly shrunken glomerulus, and beginning fibrosis surrounding the renal corpuscle. Additionally, tubular cells exhibit signs of degradation and necrosis, losing cellular details and boundaries[31]

According to Qassim[4], energy drink consumption affects oxidative enzymes and lowers blood levels of antioxidant enzymes when given in high doses to rats. This causes reactive oxygen species and oxidative stress, which causes degeneration and desquamation in renal tissues.

Fig. (2) shows histopathological examination in neonates' kidneys. When H&E-stained slices were examined under a light microscope in the control group, the normal histological structure of the renal cortex was shown. Bowman's capsules, glomerular capillaries, urine space, and renal corpuscles were all densely packed within the renal cortex. The proximal convoluted tubules, located close to the renal corpuscles, make up most of the renal cortex. They had limited lumens and were surrounded by cuboidal epithelial cells. Short cuboidal cells with rounded nuclei and less granular acidophilic cytoplasm encircled the huge lumen of the distal convoluted tubules.

When H&E-stained renal cortical tissues were examined under a light microscope in the low-dosage group, degradation was seen. The cytoplasm of certain tubular cells was vacuolated, and peritubular lymphatic infiltration was seen. The proximal and distal convoluted tubules' cell nuclei displayed partial or complete damage, indicating disintegration. It has also been discovered that the nucleus compresses. There was a large capsular gap and shrinking glomeruli: enlarged glomeruli and a smaller capsular space inside the Bowman's capsule.

When H&E-stained samples were examined under a light microscope, damage was found in the high dosage group's renal cortical sections. The cytoplasm of certain tubular cells was vacuolated. Capillary congestion and peritubular space expansion were seen. The proximal and distal convoluted tubules' cell nuclei displayed partial or

complete damage, suggesting disintegration. It has also been discovered that the nucleus shrinks. There was a wide capsular gap and decreasing glomeruli.

This finding similarity explained by Fareed[39] claimed that caffeine consumption results in kidney necrosis, congestion, or total degeneration in newborns. Interstitial hemorrhage and dilated collecting ducts were observed in the renal medulla. Prolonged high-dose coffee consumption causes tubular injury mediated by blocked proximal adenosine receptors. And Al-Basher[8] reported that newborn mice exposed to energy drinks displayed growing and degenerating glomeruli and delated urinary spaces.

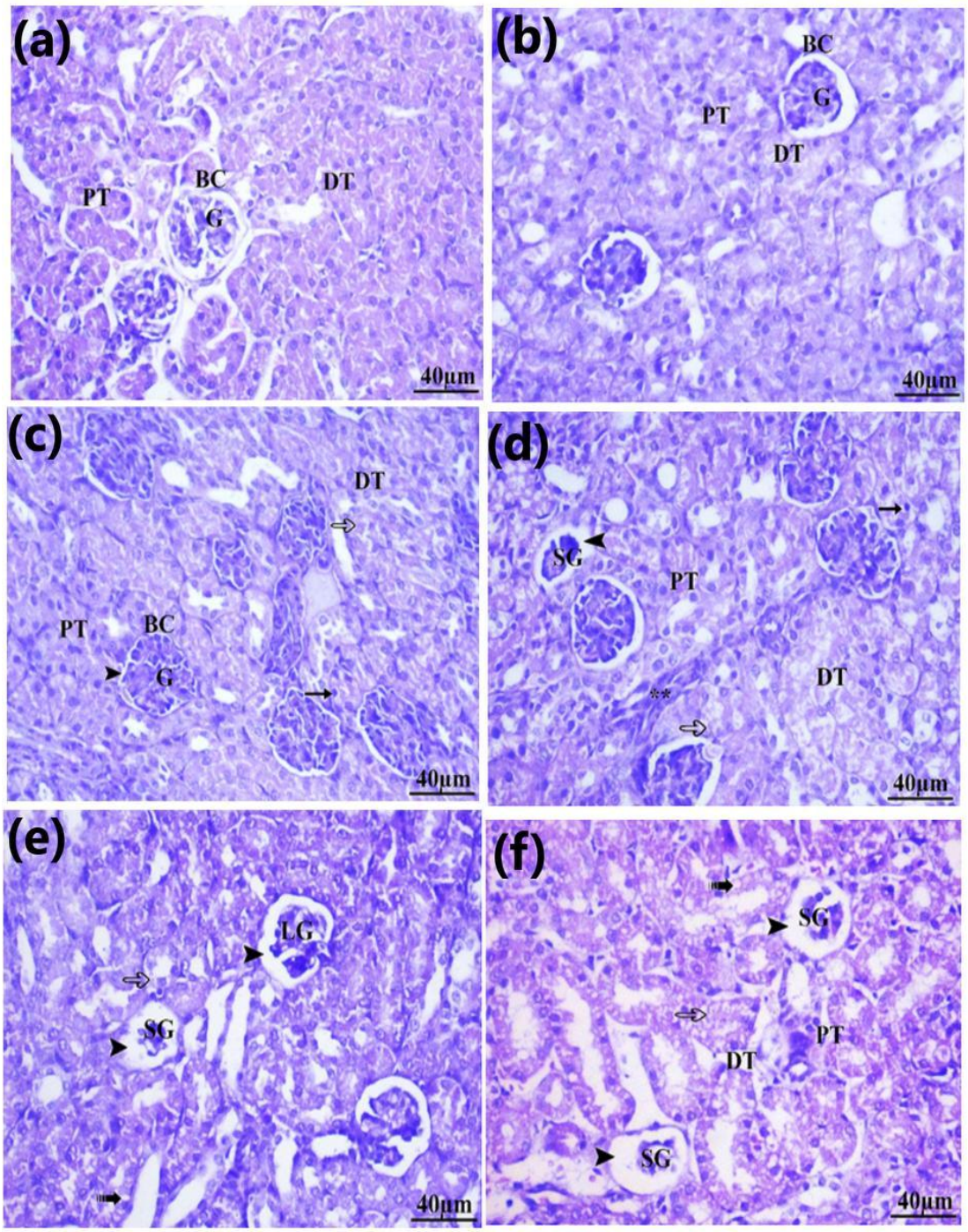

Fig. 2: Photomicrographs of a section of the kidney of neonates H&E stain, G: glomerular capillaries, BC: Bowman's capsule, arrowhead: urine space, PT: Proximal convoluted tubules, DT: distal convoluted tubules. (a), (b): control group. (c), (d): low dose group. (e), (f): high dose group

Comet assay results

Table (3) and **Fig. (3)** illustrate the findings from the comet assay for DNA damage. A comet assay was utilized to assess DNA damage in the kidney tissues of mother rats following the gestation and lactation periods and their neonates. **Table (3)** presents a statistically significant increase in the % of DNA, olive tail moment, and tail moment in the low-dose and high-dose groups compared to the control group. This could be attributed to the cytotoxicity or simple chromosomal damage caused by the ingredients in energy drinks in the alkaline comet assay, a highly sensitive tool for assessing DNA damage and repairing all levels of the individual cell[38].

Ansari[41] claimed that increased DNA fragmentation and comet tail length are indicators of DNA damage. Reactive oxygen species, can directly attack DNA, causing damage. Oxidized macromolecules, such as malondialdehyde, can also form adducts with DNA that can induce cancer and mutation. Consuming energy drinks increases oxidant stress because they produce reactive oxygen species, which upset the equilibrium between oxidative and antioxidant processes in tissues directly damage important cellular constituents like proteins, lipids, and DNA and ultimately, cell death. Oxidative damage to proteins is the main effect of ROS.[42,3].

According to Kassab and Tawfik[43], adult male albino rats given energy drinks daily for eight weeks showed signs of toxicity, including DNA damage and lipid Peroxidation (oxidative stress), which were linked to the preservatives or caffeine gradient in the energy drinks.

Table 3: The comet assay parameter for DNA damage in kidney tissues of mother rats and neonates in different studied groups.

	Mather rats tissues		
	% tail in DNA	**Tail moment**	**Olive tail moment**
Control	6.04 ± 0.57	0.62 ± 0.50	0.72 ± 0.04
Low-dose (5mL/kg)	12.07 ± 0.46 [a]	1.10 ± 0.55[a]	1.65 ± 0.05[b]
High-dose (10mL/kg)	14.20 ± 0.37[ab]	1.52 ± 0.33[ab]	1.74 ± 0.10[ab]
	Neonatal tissue		
Control	5.28 ± 0.51	0.42 ± 0.04	0.67 ± 0.04
Low-dose (5mL/kg)	7.93 ± 0.35[a]	0.61 ± 0.04[a]	1.14 ± 0.03[a]
High-dose (10mL/kg)	8.83 ± 0.52 [ab]	0.76 ± 0.04[ab]	1.27 ± 0.03[ab]

Note: data are presented as mean ± SD (n=6). Statistically significant ($p < 0.05$) differences are shown by lowercase letters.

[a] ($p < 0.05$) concerning the control group. [b] ($p < 0.05$) concerning low dose group.

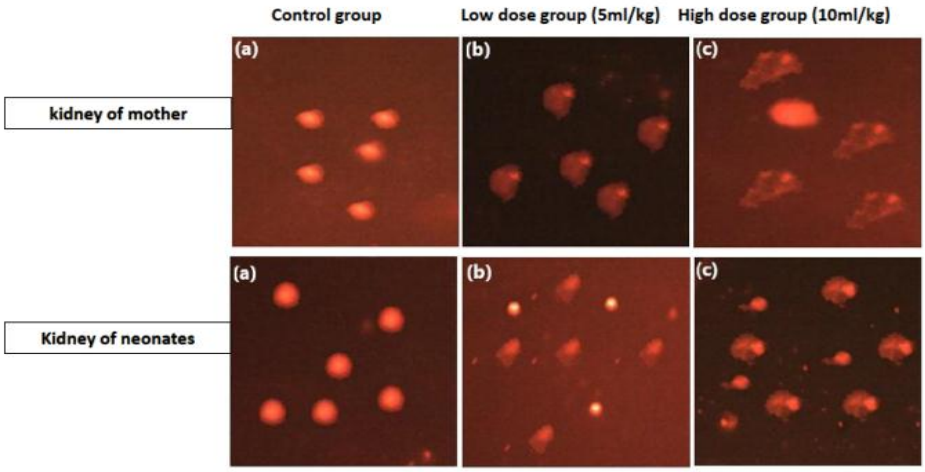

Fig. 3: Effect of energy drinks on the kidney tissue of mother rats and neonates using comet procedure in different studied groups.

(a): control group, (b): low dose group, and (c): high dose group.

Conclusion

The findings indicate that 42 days of energy drink administration in pregnant rats led to increased MDA levels and reduced SOD and GSH levels, signifying oxidative stress. A significant rise in DNA fragmentation was seen in the kidneys of the mother rats and their neonates. A histological investigation indicated that the renal tissues of the mother rats and their neonates were compromised, exhibiting congestion, destruction to cell nuclei in the high-dose group, and nuclear shrinkage. The intake of energy drinks affects neonatal renal function, resulting in increased levels of creatinine, urea, and uric acid. The study utilized an animal model; further research is recommended to investigate the effects of long-term energy drink usage on human neonates.

REFERENCES

1. I. Gheith, "Clinical Pathology of caffeinated and non-caffeinated energy drinks", ***Life Sci,*** 14(9), 21-36(2017).
2. E.Y. Elbendary, M.H. Mahmoud, S.F. Salem, A.M. Farah, "The Effects of Energy Drink Consumption on Kidney and Liver Function: A Comparative Study", ***J Bio Med***, 11(3), 171-181(2023).
3. S.B.M.Elsayed, A.M.H.Afify, W.A. Abd El-Wahab, A. A. Mohammed and R.A. Abd AlRahman, "Study of the biochemical, histological and cytogenetic effects of two different energy drinks (EDs); red bull and power horse; on brain of adult male albino rats and to determine the possible protective role of Omega-3 on the adverse effects of EDs", ***J Adv Med Med Res***, 1(2), 55-66(2020).
4. A. H.Qassim, M.A. Alsammak and A. A. Ayoob, "Histopathological changes in kidney and pancreas induced by energy drinks in adult male rats", ***I J Vete Sci***, 36(1), 111-116(2022).
5. G.I. Uwaifo, "Beware Energy Drinks: A case of a toxic triad syndrome in a diabetic patient with nonalcoholic fatty liver disease", ***Am J Med Sci,*** 358(4), 304-311(2019).
6. L.Khayyat, A.Essawy, J Sorour and M Al Rawi, "Impact of some energy drinks on the structure and function of the kidney in wistar albino rats", ***Life Sci J ,*** 11(10), 1131-1138(2014).
7. F. Olaleru and P. Odeigah, "Effects of energy drink on sperm morphology, haematological parameters and behavior of adult male mice", ***ARRB,*** 6(5), 288-296 (2015).
8. G. I. Al-Basher, H. Aljabal, R. S. Almeer, A. A. Allam and A. M. Mahmoud, "Perinatal exposure to energy drink induces oxidative damage in the liver, kidney and brain, and behavioral alterations in mice offspring", ***Biomed Pharmacother,*** 102, 798-811(2018).
9. G. E. Giles, C. R. Mahoney, T.T. Brunyé, A. L. Gardony, H.A. Taylor and R.B. Kanarek, "Differential cognitive effects of energy drink ingredients: caffeine, taurine, and glucose", ***Pharmacol Biochem Behav***, 102(4), 569-577(2012).
10. M. A. Elizabeth, O. Ebun, O. I. Roli and O. Augustine, "Caffeinated energy drink induces oxidative stress, lipid peroxidation and mild distortion of cells in the renal cortex of adult wistar rats", ***Eur J Pharm Med Res***, 7(6), 817-824(2020).
11. A. K. Wesselink, L. A. Wise, K. J. Rothman and K. A. Hahn, "Caffeine and caffeinated beverage consumption and fecundability in a preconception cohort", ***Reprod Toxicol,*** 62, 39-45(2016)
12. J. S Sisti, S. E. Hankinson, N. E. Caporaso, F. Gu, R. M Tamimi, B. Rosner, X. Xu and R. Ziegler, "Caffeine, coffee, and tea intake and urinary estrogens and estrogen metabolites in premenopausal women", ***Cancer Epidemiol Biomark Prev***, 24(8), 1174-1183(2015)
13. J. L. Temple, C. Bernard, S. E. Lipshultz, J. D. Czachor, J. A. Westphal and M. A. Mestre, "The safety of ingested caffeine: a comprehensive review", ***Front Psychiatry***, 8, 80 (2017).
14. Y.Kaya , V.Tokgöz , E. Dalyan and H. M. Tanır, "The Effect of Caffeine

Intake Levels During Pregnancy on Newborn Health", ***Eski Med ,*** 5(1), 23-28(2024).

15. A. Purkiewicz, R. P.Fiećko, F.Sörgel and M. Kinzig, "Caffeine, paraxanthine, theophylline, and theobromine content in human milk", ***Nutrients,*** 14(11), 2196(2022).
16. Organization of Economic Corporation and Development (OECD), "Guidelines on direct calculation of animals dose from humandose", ***J Nat Sci Res***, 4(18), 2014 (2000).
17. D. H. Hanna, Y. M. Al-Habashy, M. A. El Desouky, H. A. Abd El Rahman, " Impact of Energy Drinks on Hepatic and Renal Tissues in Pregnant Wistar Rats", ***Egypt J Vet Sc***i , 1-12 (2024)
18. M. Nishikimi, N.A. Roa and K. Yogi, "Colorimetric determination of super oxide dismutase", ***Biochem Bioph Res Common,*** 46, 849 – 854(1972).
19. E. Beutler, O. Duron and M.B. Kelly, "Determination of reduced glutathione in tissue homogenate", ***J Lab Clin Med,*** 61 , 882(1963).
20. J. Schirmeister, H. Willmman and H. Kiefer, "Plasma creatinine as rough indicator of renal function", ***Dtsch Med Wschr,*** 89, 1940–1947(1964)
21. J. K. Fawcett and J. E. Scott, "A rapid and precise method for the determination of urea", ***J Clin Pathol,*** 13,156-159(1960).
22. D. Barham and P. Trinder, "An improved colour reagent for the determination of blood glucose by the oxidase system", ***Analyst,*** 97(1151), 142-145(1972).
23. D. H. Hanna, S. N. Beshay, E. EL. Shafee and H. A. Abd El-Rahman,"The protective effect of aqueous extract of Stevia rebaudiana against tartrazine toxicity in male Wistar rat", ***Cell Biochem Funct,*** 41(8), 1462-1476(2023).
24. D. H. Hanna, A. K. Al-Atmani, A. A. AlRashidi, E. E. Shafee", Camellia sinensis methanolic leaves extract: Phytochemical analysis and anticancer activity against human liver cancer cells", ***PLOS ONE,*** 19(11), e0309795(2024).
25. A. A. Sayed, M M. Abdel Moteleb, E. El. Shafee, D. H. Hanna, "Oral drug delivery vehicle for insulin and curcumin based on egg albumin-dextran conjugate: In vitro and in vivo studies", ***J Drug Deliv Technol,*** 100, 106052 (2024).
26. D. H. Hanna, R.Osailan and H. A. Ahmed, "Stevia rebaudiana methanolic leaf extract in Egypt: phytochemical analysis, antioxidant, anti-lipid Peroxidation, antihemolytic, antimetastatic, and anticancer properties", ***J Food Biochem***, (14),1-22 (2023).
27. D.H. Hanna, M. M. Aziz and E. El. Shafee, "Effective-by-method for the preparation of folic acid-coated TiO2 nanoparticles with high targeting potential for apoptosis induction against bladder cancer cells (T24)", ***Appl Biochem Biotechnol,*** 70(5), 1597-1615(2023).
28. S.S. Hassan, D.H.Hanna and S.S. Medany, "The double-edged sword of the amoxicillin antibiotic against prostate cancer in nano palladium form and its electrochemical detection of dopamine", ***Appl Organomet Chem***, 37(4), e7026 (2023).
29. R.Reis, M. Charehsaz, H.Sipahi, A.I.D. Ekici, Ç. Macit, H. Akkaya and A. Aydın, "Energy drink induced lipid peroxidation and oxidative damage in rat liver and brain when used alone or combined with alcohol", ***J Food Sci***, 82(4), 1037-1043((2017).
30. G. Richards and A. P. Smith, "A review of energy drinks and mental health, with a focus on stress, anxiety, and depression", ***J Caffeine Res***, 6(2), 49-63 (2016).
31. W. Mansy, D.M. Alogaiel, M.H. anafi and E.Zakaria,"Effects of chronic consumption of energy drinks on liver and kidney of experimental rats", ***Trop J Pharm Res***,16(12), 2849-2856(2017).
32. M. Sgorbini, F. Bonelli, A. Rota, P. Marmorini, G. Biagi, M. Corazza

and A. Pasquini, "Maternal and neonatal evaluation of derivated reactive oxygen metabolites (d-ROMs) and biological antioxidant potential in the horse", ***Theriogenology,*** 83(1), 48-51(2015).
33. K. S. Frazier,"The impact of functional and structural maturation of the kidney on susceptibility to drug and chemical toxicity in neonatal rodents", ***Toxicol Pathol,*** 49(8), 1377-1388(2021)
34. S. Perrone, M.L.Tataranno, G.Stazzoni and G .Buonocore, "Oxidative stress and free radicals related diseases of the newborn", ***Adv Exp Med Biol***, 3, 1043-1050 (2012).
35. H.M. Eltahir, G. Alamri, A. Alamri, A. Aloufi, M. Nazmy, H. Elbadawy, S. Bahashwan, H. M. Elbadawy , Y.M. Alahmadi and M. Abouzied, "The metabolic disorders associated with chronic consumption of soft and energy drinks in rats", ***Acta Biochim Pol,*** 67(1), 79-84(2020).
36. I. M. Muxiddinovna**,** "Impact of energy drinks and their combination with alcohol to the rats metobolism", ***Gospodarka i Innowacje***, 22, 2545-0573 (2022).
37. I. S. Akande and O.A.Banjoko, "Assessment of biochemical effect of "Power Horse" energy drink on hepatic, renal and histological functions in Sprague Dawley rats", ***ARRB***,1(3), 45-56(2011).
38. S.D. Khalaf, "The Genotoxicity and Health Effects of Stimulant (Energy) Drinks", ***Ann Rom Soc Cell Biol***, 27(1), 193-198(2023).
39. S.A. Fareed and H.E. Mostafa, "Could aspartame exacerbate caffeine effects on renal maturation in rat's offspring? A biochemical and histological study", ***Birth Defects Res***, 113(1), 90-107(2021).
40. E. Greene, K. Oman and M. Lefler, "Energy Drink–Induced Acute Kidney Injury", ***Ann Pharmacother***, 48(10), 1366-1370 (2014)
41. F.A. Ansari, S. N. Ali, A. A.Khan and R. Mahmood, "Acute oral dose of sodium nitrite causes redox imbalance and DNA damage in rat kidney", ***J Cell Biochem,*** 119(4), 3744-3754(2018).
42. A.M. Ahmed, "Expression of transcription factor NF-KAPPA B/P65 and cyclooxygenase-2 (COX-2) in testicular damage induced by Red Bull energy drink in rat", ***Int J Adv Res (Indore)***, 3(10), 49-56(2016).
43. A. A. Kassab and S. M .Tawfik, "Effect of a caffeinated energy drink and its withdrawal on the submandibular salivary gland of adult male albino rats: A histological and immunohistochemical study", ***J Egypt Public Health Assoc***, 41(1), 11-26(2018).

Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 1379-1392.

نشرة العلوم الصيدلية
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

التاثير السلبي لمشروبات الطاقة علي انسجة الكلي لإناث جرزان ويستار الحامل وصغارها

هبة علي عبد الرحمن[١] – آية رجب عبد الغني[٢] – محمد أ. الدسوقي[٢] – دميانة حنا[٢]*

[١]قسم علم الحيوان، كلية العلوم، جامعة القاهرة، القاهرة، مصر

[٢]قسم الكيمياء، كلية العلوم، جامعة القاهرة، القاهرة، مصر

شهد تصنيع مختلف مشروبات الطاقة، التي تهدف إلى اجتذاب الأفراد بتزويدهم بطفرة من الطاقة على مدار اليوم ارتفاعا كبيرا ومع ذلك، يمكن لمشروبات الطاقة أن تحدث آثارا صحية ضارة مختلفة على الأفراد. تهدف الدراسه الي دراسه الاثار السلبيه المحتمله لمشروبات الطاقه علي أنسجة الكلى لجرذان ويستار الحامل وصغارها. تم تقسيم المجموعات الدراسية الي ثلاثة فئات: المجموعةاه الآولي، حصلت علي مياه مقطرة. وحصلت المجموعة الثانية، وهي المجموعة ذات الجرعة المنخفضة، على ٥ مليلتر/كيلوغرام من مشروبات الطاقة. وتلقت المجموعة الثالثة، وهي المجموعة ذات الجرعة العالية، ١٠ مليلتر/كيلوغرام من مشروبات وبالإضافة إلى ذلك، فإن تقييم الضرر المؤكسد يشمل قياس مستويات مالونديالديهايد والمضادات للاكسدة مثل SOD,GSH أجري تقييم لأداء الكلى، وتحديدا الكرياتينين وحمض اليوريك واليوريا وأجري تحليل اختبار الكومت و فحص الأنسجة. اسفر استهلاك مشروبات الطاقه علي امهات الجرزان الحامل وصغارها ارتفاع ملحوظ في مستويات مالونديالديهايد وانخفاض مستويات SOD و الجلوتاثيون بالإضافة الي ذلك حدوث تغيير في الحمض النووي و تغيير في انسجة الكلي ، بينما حدث تغير طفيف في وظائف كلي جرزان الام الحامل. وتبين أنه تناول مشروبات الطاقة في فترتي الحمل والرضاعة له آثار ضارة علي جرزان الأم الحامل وصغارها ولذلك فإن هذة مساله صحية خطيرة تحتاج الي معالجة.