

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Impact of Energy Drinks on Hepatic and Renal Tissues in Pregnant Wistar Rats

Demiana H. Hanna1*, Yasmin M. Al-Habashy¹ , Mohamed A. El-Desouky¹ and Heba Ali Abd El‐**Rahman²**

¹ Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt

²Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt

Abstract

OPULAR non-alcoholic beverages that are consumed worldwide are energy drinks. Energy drinks' high caffeine content stimulates the central nervous system and heart. Energy drinks can cause hepatitis, liver damage, and an increase in the extraction of water and sodium. The **D**OPULAR non-alcoholic beverages that are consumed worldwide are energy drinks. Energy drinks' high caffeine content stimulates the central nervous system and heart. Energy drinks can cause hepatitis, liver damage, and an study included three groups of pregnant rats: The control group received distilled water, the second group received low-dose energy drinks (5 ml/kg), and the third group received high-dose energy drinks (10 ml/kg). The findings demonstrated a significant rise (p <0.05) in the malondialdehyde (MDA) concentration and a substantial decrease in the levels of antioxidant enzymes in both treatment groups compared to the control group. Furthermore, the treated groups' liver and renal function parameters significantly increased. Energy drinks triggered histopathological changes in the liver and kidney tissues. The consumption of energy drinks during pregnancy could have a substantial detrimental effect on the health of pregnant rats.

Keywords: Energy drinks, Pregnancy, Liver damage, Kidney damage, Wistar rats.

Introduction

Energy drinks are slightly carbonated, non-alcoholic beverages made with substances to increase energy, especially caffeine, to provide the consumer with energy [1]. Energy drink consumption that contains caffeine is expanding quickly. In 1960, several energy drink firms emerged in response to customer demand in Europe and Asia. Due to the belief that energy drinks can improve physical strength, speed up reactions, reduce the need for sleep, and keep the body alert with a greater mental focus, their consumption has increased globally¹. Typically, they contain sugar, salt, guarana, taurine, caffeine, and vitamin B6 [2]. Caffeine (1,3,7-trimethyl Xanthine) is crucial since it stimulates the central nervous and cardiovascular system. Energy drinks are sold in cans of 250 ml (equivalent to 80 mg of caffeine) or 355 ml (equivalent to 113.6 mg of caffeine). They have an approximate caffeine content of 32 milligrams for

every 100 milliliters. Soft beverages, on the other hand, have about 40 mg of caffeine per can [3]. Energy drinks can have adverse effects such as low academic success [4], aggressive conduct, substance misuse [4], tension, anxiety [5], elevated pulse rate, elevated both the diastolic and systolic blood pressure [6], Type 2 diabetes, obesity, and overweight risk [7], microvascular injury to the kidneys and faster progression of chronic kidney disease [8], dental decay [7], hepatic impairments [3], lipid metabolic changes [4], hepatitis [9] and increased extraction of sodium and water [10], headaches, tummy aches, irritability, exhaustion, and unsatisfactory sleep [4]. Recent research has indicated that energy drinks may contribute to oxidative stress in several organs [11,12], reduced antioxidant defenses and heightened reactive oxygen species (ROS) production can harm proteins, lipids, nucleic acids and potentially cause cell death [13].

*Corresponding author: Hanna, Demiana Badry, E-mail[: dhelmy@sci.cu.edu.eg](mailto:dhelmy@sci.cu.edu.eg) , Tel.: 01015181160 (Received 04 June 2024, accepted 30 July 2024)

DOI: 10.21608/EJVS.2024.295316.2145

[©]National Information and Documentation Center (NIDOC)

Associated with the physiological alterations that take place during pregnancy, pregnant women's exposure to toxins may differ from non-pregnant women's exposure to the same dose. The effects of pregnancy on gastrointestinal absorption are profound, this is caused by pregnancy's hormonal impacts, as the emptying of the stomach takes longer, reduced gastrointestinal motility and the duration of transit is increased. These changes cause absorption to be more thorough but delayed [14]. Additionally, the pharmacokinetics of toxins alter during pregnancy. Most drugs exhibit an increase in volume of distribution due to the accumulation of body fat and increased plasma volume. Because of the decrease in albumin levels and increase in cardiac output during pregnancy, more free medicine can reach target organs like the placenta [15].

Changes in serum pH during pregnancy can impact the ionization of some medications, leading to modifications in tissue absorption and excretion. Early in pregnancy, the fetal pH is greater than the mother's pH. Although weak acids pass through the placenta in an electrically neutral state, the more alkaline fetal fluids may ion trap them. Late in pregnancy, maternal free fatty acids increase and cause serum proteins to lose their binding to protein-bound medications. Because the natural "chelator" function of these proteins is reduced, this displacement may result in increased toxicity. On the other hand, a pregnant woman's hyperdynamic state may signal an increase in glomerular filtration rate, which could result in the urine containing more substances that have been cleared by the kidneys [16].

Numerous studies have been conducted on the relationship between female fertility and caffeine, with varying degrees of success. The majority of studies have been retrospective in nature, looking at caffeine intake in relation to a woman's stage of pregnancy. They have either displayed decreased fecundity or no fertility at all [17]. Although the exact mechanisms are unknown, caffeine or other components of particular energy beverages may affect fertility. Changes in estradiol and other hormones have also been connected to caffeine [18]. During pregnancy, the metabolism of caffeine decreases, the placenta readily absorbs caffeine and its metabolites, and exposing the developing fetus to prolonged caffeine exposure [19].

The correlation between energy drinks and pregnant women remains poorly comprehended despite prior indications of an association with public health. The present study aims to determine the potential effects of energy drinks on pregnant rats. Glutathione reductase, malondialdehyde, and superoxide dismutase were measured in the liver and kidney tissues to assess oxidative markers. In addition, the liver enzymes alanine aminotransferase and aspartate aminotransferase, as well as kidney function indicators such as urea, uric acid, and creatinine, were analysed biochemically. The extent of DNA degradation was also assessed, and alterations in the histology of liver and kidney tissues were examined.

Materials and Methods

Chemicals

Energy drinks were purchased from a nearby grocery in Cairo, Egypt, along with all laboratory reagents and fine chemicals from Biodiagnostic (Doki, Giza, Egypt). Every other material employed as a reagent or chemical was of a quality that could be sold commercially.

Study design and animals

The National Organization for Drug Control and Research's (NODCAR) animal house provided 9 males and 18 females Wistar albino rats (*Rattus norvegicus*) to use in this study. They were raised at a laboratory animal unit of Cairo University's Faculty of Science, Zoology Department, weighing between 160 and 180 g. All animals were housed in 65 \times 25 \times 15 cm polypropylene cages with free food and water access, a 12-hour cycle of light and dark, controlled temperature (20–23 °C), and tracked humidity (40– 50%). The animals were allowed one week in their housing to acclimate to the experimental settings.

Rats were individually housed for mating purposes, with two female rats placed in a cage overnight with a single male rat. Vaginal smears were collected from the female rats and examined the next day. A small amount of saline solution was injected into each rat's vaginal opening, and the resulting vaginal fluid containing cell suspension was placed on a slide. Two droplets of this suspension were mixed with 0.1% methylene blue and examined under a microscope at 100x magnification after drying to determine the presence of sperm, indicating gestation day 0 [20]. Three groups, each consisting of six pregnant rats, were assigned as follows: The control group received an oral dose of distilled water in a volume equivalent to the energy drink doses. The low-dose group was orally administered the energy drink at a dose of 5 ml/kg [21]. The high-dose group received an oral gavage of the energy drink at a dose of 10 ml/kg [21]. The energy drinks were administered from gestation day 5 to day 19, totaling 15 days of exposure. Euthanasia of the rats was performed by administering an overdose of sodium pentobarbital at a minimum dosage of 100 mg/kg body weight.

Specimens' collection

Following a heart puncture, blood was drawn out and transferred into plain tubes to enable coagulation. Subsequently [22], the blood was centrifuged at 3000 revolutions per minute for fifteen minutes [22]. The serum that was recovered was stored at -20[°]C for further examination.

Every rat had its abdominal wall cut along the mid-ventral line, extending the whole abdominal cavity. Subsequently, after removing the liver and kidney tissues, they were cleaned with saline solution, and divided into two sections. A part of it was kept at -20°C for biochemical study, while another was kept for histological examination in 10% formalin.

Biochemical analysis

After centrifugation, the serum was used to assess various parameters, including urea, creatinine, and uric acid, for kidney function evaluations alanine amino transferase (ALT) and aspartate amino transferase (AST)for liver function evaluations.

The levels of urea and creatinine in all sample sera were determined using the Urease-Berthelot Method [23] and Colorimetric Kinetic Method [24], respectively, with the bio diagnostic test kits (CAT. No. UR 21 10) and (CAT. No. CR 12 51). Furthermore, the uric acid concentration (CAT. No. UR 21-20) was measured using the enzymatic colorimetric method [25]. In addition, the biodiagnostic kits (CAT. NO. ALT 10 31 (45)) and (CAT. No. AST 10 61 (45)) were employed according to the protocols above to quantify the levels of ALT and AST utilizing a colorimetric method [25]. It purchased the complete range of bio diagnostic kits from the Bio Diagnostic Company, Dokki, Giza, Egypt.

Oxidative stress markers evaluation

The measured the activities of malondialdehyde (MDA) [25], super oxide dismutase (SOD) [25] and reduced glutathione (GSH) [26] using the tissue extract utilizing a colorimetric approach using Biodiagnostic kits (CAT. No. MDA 25 29), (CAT. No. SOD 25 21), and (CAT. No. GSH 25 11), by the previously published procedures.

Histological examination

The samples were preserved for 48 hours in 10% neutral buffered formalin at ambient temperature. The tissues underwent an overnight soak in running tap water and were dehydrated in increasing concentrations of alcohol (absolute alcohol, 70, 80, 90, and 95%), cleaned in xylene, infiltrated in three variations of melted paraffin at 60°C for four hours, and then manually embedded in newly melted paraffin. The blocks of paraffin were sealed and refrigerated. Sections were cut to five microns in thickness using a rotary microtome, mounted on glass slides, dried at 37 °C, and carefully preserved for staining with hematoxylin and eosin before being examined in a bright field. Bright microscope [25,27].

Comet assay

Single-cell gel electrophoresis (SCGE), also Known as the comet assay, is an often employed method for determining DNA damage in specific cells. cellular DNA damage, such as strand breaks and fragments, was segregated from whole DNA when subjected to an electrophoretic field, resulting in a comet-tail structure visible under a fluorescence microscope. This approach has already been described. After treatment liver and kidney tissues, examine the slides with 20 μg/mL of ethidium bromide under a fluorescence microscope. For instance, a computerized image analysis system captured 100 random comet shapes on each slide. The system then used TriTek Comet ScoreTM software (TriTek Corp.) to analyze the photos and calculate the parameters of the comet. Tail moments and tail DNA are popular measures created to assess the outcomes. Concerning DNA mobility and abundance in the tail, it is believed that the Olive tail moment (OTM) parameter is the most effective way to determine DNA damage. It is calculated utilizing the subsequent formula (1) [28-31]:

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

Statistical analysis

SPSS Statistics 25 was used to do the statistical analysis. The means \pm standard deviations (SD) were used to express all the data. A one-way ANOVA was utilized to compare the groups, followed by Tukey's post-hoc least significant difference (LSD) analysis. P values deemed significant were those with a 0.05 or below $(n = 6)$.

Result and Discussion

Biochemical assays

The findings about how energy drinks affect kidney and liver function. The treated groups have noticeably greater levels $(p<0.05)$ of serum urea, uric acid, ALT, and AST than the control group. Serum creatinine levels in the energy drink groups increased, but not significantly more than in the control group (Table 1).

The present investigation corroborated earlier findings since the energy drink-treated groups had increased AST and ALT values in comparison to the control group [32], where pregnant rats' ALT and AST enzyme levels increased in response to varying amounts of energy drinks consumed during the gestation stage. According to earlier research, energy drinks may produce too many reactive oxygen species (ROS), cause lipid peroxidation to tear down cell membranes, disrupt the plasma membrane, and cause excessive ALT and AST enzymes to leak into blood [32,33]. Also, consuming beverages with added sugar increases the chance of increased ALT [34]. Carbohydrates with a high glycemic index present in high-energy, unhealthy diets and high levels of sugars in energy drinks, such as fructose and sucrose have been associated with elevated liver enzymes and liver disorders [33,35]. Consuming fructose can stimulate de novo lipogenesis while preventing fats from being beta-oxidized in the mitochondria, which raises the amount of fat in the liver and elevates liver enzyme levels [36].

Pregnant Wistar rats that consumed energy drinks showed markedly elevated renal function (urea and uric acid). Similar findings in the past demonstrated that energy drinks, both at low and high dosages, harmed kidney function [32]. Caffeine is responsible for these changes because it inhibits the A2A adenosine receptor, causes proteinuria, speeds up the onset of interstitial inflammation, and modifies the structure and function of the kidneys [32,37]. They also found that caffeine markedly increased blood urea nitrogen levels, xanthine oxidase activation and increased H_2O_2 generation, uric acid, and superoxide anion. By interacting with O_2 , H_2O_2 created free radicals [38]. Urea's stimulation of xanthine oxidase,

which accelerates xanthine's conversion to uric acid, provides an additional explanation for the variation in these parameters. Nevertheless, the present study did not observe any noteworthy alterations, which could be attributed to the comparatively shorter trial period employed in the present investigation.

Oxidative stress markers

Oral administration of energy drinks in low and high-dose groups resulted in a substantial rise $(p \le 0.05)$ in MDA levels and a notable decline $(p \le 0.05)$ in SOD and GSH in the tissues of the kidney and liver when compared to the untreated group (Tables 2 and 3).

An imbalance between prooxidant and antioxidant levels with an excess of pro-oxidants in cells, tissues, and organs is known as oxidative stress. Numerous diseases, including diabetes, cancer, and cardiovascular ailments, are thought to have their origins in oxidative stress. ROS may be advantageous for cellular signaling and defending against microbes in cells. An imbalance between antioxidants and oxidants may be the consequence of elevated ROS generation, or decreased antioxidant defense [22,39] Elevated levels of ROS can potentially harm lipids directly. The endoplasmic reticulum, peroxisomes, mitochondria, and plasma membrane are the primary locations where endogenous ROS are produced. The most prevalent ROS that have the ability to greatly affect lipids are hydroperoxyl radical (HO•2) and hydroxyl radical (HO•), which can also harm DNA structure and proteins [40]. Using energy drinks produces ROS that damages the integrity of cellular components, including membrane lipids. MDA is created as a byproduct of lipid peroxidation, which is harmful to the cell membrane and reduces the activity of enzymes, ion exchange, and membrane permeability [7].

The rats' exposure to energy drinks elevated their levels of oxidative stress. That was demonstrated by the notable declines in SOD and GSH activity brought on by energy drinks.

The rats' exposure to energy drinks elevated their levels of oxidative stress. That was demonstrated by the notable declines in SOD and GSH activity brought on by energy drinks. These enzymes are significant antioxidants that prevent oxidative damage from free radicals in cells by cooperating with the nonenzymatic antioxidant system [32,41]. In actuality, antioxidant enzymes are the first line of defense that keeps cells safe from harm brought on by oxidative stress. Superoxide dismutase (SOD) breaks down the highly reactive superoxide anion into hydrogen peroxide, which decomposes into water by glutathione synthase (GSH) [42,43]. Research has demonstrated that high caffeine exposure causes a prooxidant state which raises the rate of protein oxidation in human cells [32,42]. Caffeine has been shown to raise BUN levels, activating xanthine oxidase dramatically. Afterwards, this process encourages the synthesis of H_2O_2 , superoxide anion, and uric acid from xanthine. Free radicals are created when H_2O_2 and O_2 mix. [34,44]

There is a study has shown that long-term caffeine use is effective in lowering lipid peroxidation and boosting antioxidants [45]. Additionally, taurine has demonstrated antioxidant action in vivo and in vitro radical scavenging [46,47]. In the research of Valle [44], adult rats' brain antioxidant enzyme activity was lowered after receiving taurine for 28 days. Additionally, animals given a combination of taurine and caffeine displayed decreased antioxidative defenses and a state of oxidative stress [44]. Thus, it is reasonable to believe that taurine and caffeine together can cause oxidative stress, which could account for the higher levels of lipid peroxidation and lower levels of antioxidants of the newborn mice in this investigation [7].

Because energy drinks have a high sugar index, they create oxidative stress that leads to prolonged hyperglycemia and excessive production of peroxide free radicals. causing interstitial inflammation, elevated proteinuria, and histological alterations, which are signs of oxidative damage [2].

Comet assay

A comet test was used to evaluate DNA damage; the parameters of this comet test for harm to DNA are shown in (Table 4 and Figure 1,2). Oral administration of varying amounts of energy drinks resulted in a non-significant increase ($p < 0.05$) in the olive tail moment in contrast to the group under control.

Following the administration of energy drinks to pregnant rats, no indications of DNA damage were seen based on the results of the comet assay. Additionally, older mice given caffeine showed less DNA damage in their peripheral blood [48]. Furthermore, taurine demonstrated geno-protective properties against arsenic-induced DNA damage in an animal experiment [49]. The outcomes in both situations support our findings, which indicate that either caffeine or taurine alone or in combination did not raise the amount of DNA damage in rats' peripheral blood.

Since this study's trial duration was shorter than that of earlier reports, it is possible that no appreciable alterations were found.

Histopathological investigations

Liver tissues

There were no histological changes to the control rat's normal anatomy of the hepatic lobules, sinusoids with a precise central vein, and hepatocyte arrangement. But in the group receiving a modest dose, induced liver tissue alternation, there were degraded cells with cytoplasmic vacuoles, altered lobular organization and nuclear deterioration, distortion of normal hepatocytes, and minor fatty degeneration. Around portal veins, a moderate invasion of lymphocytes was found. Several central veins were enlarged, and sinusoids were dilated. The necrotic region was also observed.

In the high-dose group, hepatic histology showed hydropically deteriorated cells, changed lobular configuration and nuclear disintegration in specific locations, disarrangement of typical hepatic cells, and moderate fatty degeneration. It was found that the major vein of the liver and portal vein were swollen and congested. The degradation of hepatic cells was seen. In the portal region, lymphocyte proliferation was detected. The liver histopathology analysis images are displayed in (Figure 3).

The photomicrographs of this tissue showed lesions consistent with the pattern of changes shown in liver function tests in rats given different amounts of energy drinks. The lesions that have been detected likely result from the harmful effects of energy drinks. It is conceivable that oxidative stress from energy drinks caused tissue damage, which resulted in the lesions. These findings align with a prior study that found that rats given various energy drinks showed signs of hepatotoxicity and changes in liver ultrastructure [50]. In another study, the liver tissue lesions were linked to a possible interaction between taurine and other active ingredients in energy drinks, like caffeine [32]. In addition, researchers discovered that rats given energy drinks exhibited hepatic cytoplasmic vacuolations because of lipid droplets, which were linked to degenerative alterations in hepatocytes [50]. Researchers have seen pyknosis, uneven outlines, and many mitotic figures in the nuclei of hepatocytes [50]. These alterations could be linked to the harmful impacts of preservatives such as sodium benzoate that are added to energy drinks and the toxic effects of caffeine [50].

Kidney tissues

The histological examination of the rats' kidney sections as seen in (Figure 4). Bowman's capsules, glomerular capillaries, urine space, and renal (Malpighian) corpuscles were present in the control group's renal cortex. The cubical cells with spherical nuclei encircled the tiny lumen found in the proximal convoluted tubules. Simple cubical cells with spherical nuclei in the center or at the tip of the tubule walls encircle the large lumen of the distal convoluted tubules.

Moreover, in the low-dose group, there was glomerular size and shape variation, with specific glomeruli shrinking due to atrophied glomerular tufts. Many glomeruli were fragmented and congested. The necrosis to whole or partial cell nuclei was observed in both the distal and proximal convoluted tubules, indicating dissolution. In addition, the nucleus has been found to condense, and other cells have been shown to contain vacuoles. There was invasion of inflammatory cells in the injured tubules and corpuscles of the kidney. Within the tubular cells, cellular debris was discovered. Congestion was seen between the renal tubules.

In the high-dose group, several tubular epithelial cells in renal convoluted tubules demonstrated deteriorating alterations. Several glomeruli within Bowman's capsules were enlarged and had large capsular space. Some glomerular tufts were shown to be vacuolated and shrunk. There were numerous pyknotic nuclei in tubular epithelial cells. Near blood vessels, moderate lymphatic invasion and intratubular mononuclear cell infiltration were seen. Also, congestion was observed. Many tubules exhibited a luminous cast. It was discovered that renal tubules had disrupted epithelial linings and degraded cells. The lumen of several distal tubules was expanded.

Kidney lesion may result from consuming large amounts of energy drinks because of their various components. That is explained by the fact that hazardous substances that are eliminated or cleaned during removal or withdrawal impact the renal tubules [42]. The microcirculatory problems that may be causing the intertubular inflammation and hemorrhage zones could be caused by the caffeine in energy drink [37]. Under an electron microscope, the cells of the proximal and distal tubules and the renal

corpuscles' nucleoli and cytoplasmic organelles showed ultrastructural alterations after "Power Horse" drinks administration [37]. Rats exposed to different quantities of energy drinks for 12 weeks showed differences in renal function that were inconsistent with the damage in these tissues' histopathology [32]. Damage was caused by energy drinks' oxidative stress, leading to tissue damage. The possible interaction between taurine and caffeine caused lesions in the renal tissues [42]. Red Bull consumption negatively impacted the standard renal cortex histological structure in rats. In the group that used energy drinks, the renal cortex displayed histological changes in H&E-stained sections. These changes included segmentation of glomerular capillaries, which appeared dilated and crowded, glomeruli degenerating as Bowman's space dilated, renal tubules that were significantly distorted and dilated, with sloughing necrotic cells inside and pyknotic nuclei and cytoplasmic vacuoles in their lining epithelial cells [51].

Conclusion

The findings of this study suggest that oral energy drinks at both low and high doses during pregnancy can lead to notable pathological lesion in the liver and kidneys in rats, potentially contributing to the decline in their overall function. Energy drinks can have adverse side effects, such as a notable reduction in antioxidant enzyme levels and an increase in lipid peroxidation products, liver enzymes, and markers of kidney function. These findings indicate the need for a thorough assessment of the security of energy drinks for individuals, focusing on pregnant women. Hence, the conclusions drawn from this research will apply to larger organisms, animal models of different species, and even to people.

Note: Data are presented as mean \pm SD of six values in each group (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 the low-dose group.

TABLE 2. Effect of Energy drinks on oxidative stress markers in liver tissue of the tested groups (low and high doses groups) compared to the control group.

Note: Data are presented as mean \pm SD of six values in each group (n = 6). Statistically significant (p < 0.05) differences are shown by lowercase letters—abbreviations: GSH, glutathione reductase; MDA, malondialdehyde; SOD, superoxide dismutase.

a ($p < 0.05$) concerning the control group.

b (p <0.05) concerning the low-dose group.

TABLE 3. Effect of Energy drinks on oxidative stress markers in kidney tissue of the tested groups (low and high doses groups) compared to the control group.

Note: Data are presented as mean \pm SD of six values in each group (n = 6). Statistically significant (p < 0.05) differences are shown by lowercase letters—abbreviations: GSH, glutathione reductase; MDA, malondialdehyde; SOD, superoxide dismutase.

a (p <0.05) concerning the control group.

b ($p < 0.05$) concerning the low-dose group.

TABLE 4. The comet assay parameters for DNA damage on liver and kidney tissues in the different studied groups.

Note: Data are presented as mean \pm SD of six values in each group (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

Fig. 1. Fluorescence microscopic pictures stained by ethidium bromide of the liver tissue nucleus in the low-dose group (b) and the high-dose group (c), compared with the nucleus in the control group (a), show the intact nucleus in all studied groups without any tail formation. All images are at 40X magnification.

Fig. 2. Fluorescence microscopic pictures stained by ethidium bromide of the kidney tissue nucleus in the low-dose group (b) and the high-dose group (c), compared with the nucleus in the control group (a), show the intact nucleus in all studied groups without any tail formation. All images are at 40X magnification.

Fig. 3. Histological examination of the liver stained by H&E. Low dose group (c&d) and High dose group (e&f), compared with the control group (a&b). central vein (CV), hepatocyte (H), sinusoids (S), Kupffer cell (thin arrow), portal vein (PV), bile duct (B), moderate invasion of lymphocytes (LI), necrotic region (N), nuclear deterioration (notch arrow), degradation of hepatic cells (wavy arrow), hydropic deteriorated cells (hollow arrow) moderate fatty degeneration (dotted arrow). All images are at 800X magnification.

Fig. 4. Histological examination of the kidney stained by H&E. Low dose group(c&d) and High dose group (e&f), compared with the control group (a&b). glomerular capillaries (G), Bowman's capsules (BC), and urine space (arrowhead). Distal (DT), proximal (PT), pyknotic nuclei (wavy arrow), cells contain vacuoles (curved arrow), dissolution (notch arrow), cellular debris (triangle), Shrink glomeruli (SG), capsular space (hollow arrow). Intratubular mononuclear cell infiltration (dotted arrow), Lymphocyte infiltration (LI), hydropic degeneration (bold arrow), large glomeruli (LG). All images are at 800X magnification.

 $40 \mu m$

References

- 1. Tóth, Á., Soós, R., Szovák, E., M. Najbauer, N., Tényi, D., Csábí, G. and Wilhelm, M. Energy drink consumption, depression, and salutogenic sense of coherence among adolescents and young among adolescents and young adults. *International Journal of Environmental Research and Public Health*, **17**(4), 1290 (2020).
- 2. Richards, G. and Smith, A. P. A review of energy drinks and mental health, with a focus on stress, anxiety, and depression. *Journal of Caffeine Research*, **6**(2), 49-63 (2016)
- 3. Corbo, M. R., Bevilacqua, A., Petruzzi, L., Casanova, F. P. and Sinigaglia, M. Functional beverages: the emerging side of functional foods:

commercial trends, research, and health implications. *Comprehensive Reviews in Food Science and Food Safety*, **13**(6), 1192-1206 (2014).

- 4. Holubcikova, J., Kolarcik, P., Madarasova Geckova, A., Reijneveld, S. A. and van Dijk, J. P. Regular energy drink consumption is associated with the risk of health and behavioural problems in adolescents. *European Journal of Pediatrics*, **176**, 599-605 (2017).
- 5. Wikoff, D., Welsh, B. T., Henderson, R., Brorby, G. P., Britt, J., Myers, E. and Doepker, C. Systematic review of the potential adverse effects of caffeine consumption in healthy adults, pregnant women, adolescents, and children. *Food and Chemical Toxicology*, **109**, 585-648 (2017).
- 6. Grasser, E. K., Yepuri, G., Dulloo, A. G. and Montani, J. P. Cardio-and cerebrovascular responses to the energy drink Red Bull in young adults: a randomized cross-over study. *European Journal of Nutrition*, **53**, 1561-1571 (2014).
- 7. Al-Basher, G. I., Aljabal, H., Almeer, R. S., Allam, A. A. and Mahmoud, A. M. Perinatal exposure to energy drink induces oxidative damage in the liver, kidney and brain, and behavioral alterations in mice offspring. *Biomedicine and Pharmacotherapy*, **102**, 798-811 (2018).
- 8. Schalkwijk, C. G. and Stehouwer, C. D. A. Methylglyoxal, a highly reactive dicarbonyl compound, in diabetes, its vascular complications, and other age-related diseases. *Physiological Reviews*, **100**(1), 407-461 (2020).
- 9. Vaillant-Roussel, H., Cadwallader, J. S. and Gelly, J. Sugar, the taste of good times. *Exercer la Revue Francophone de Medecine Generale*, **26**(122), 302- 302 (2015).
- 10. Spaeth, A. M., Goel, N. and Dinges, D. F. Cumulative neurobehavioral and physiological effects of chronic caffeine intake: individual differences and implications for the use of caffeinated energy products. *Nutrition Reviews*, **72**(suppl_1), 34-47 (2014).
- 11. Reis, R., Charehsaz, M., Sipahi, H., Ekici, A. I. D., Macit, Ç., Akkaya, H. and Aydın, A. Energy drink induced lipid peroxidation and oxidative damage in rat liver and brain when used alone or combined with alcohol. *Journal of food Science*, **82**(4), 1037-1043 (2017).
- 12. Díaz, A., Treviño, S., Guevara, J., Muñoz-Arenas, G., Brambila, E., Espinosa, B. and Aguilar-Alonso, P. Energy drink administration in combination with alcohol causes an inflammatory response and oxidative stress in the hippocampus and temporal cortex of rats. *Oxidative Medicine and Cellular Longevity*, **2016**(1), 8725354 (2016).
- 13. Sadiq, I. Z. Free radicals and oxidative stress: Signaling mechanisms, redox basis for human diseases, and cell cycle regulation. *Current Molecular Medicine*, **23**(1), 13-35 (2023).
- 14. Zelner, I., Matlow, J., Hutson, J. R., Wax, P., Koren, G., Brent, J. and Finkelstein, Y. Acute poisoning during pregnancy: observations from the Toxicology Investigators Consortium. *Journal of Medical Toxicology*, **11**(3), 301-308 (2015).
- 15. Nitsche, J. F., Patil, A. S., Langman, L. J., Penn, H. J., Derleth, D., Watson, W. J. and Brost, B. C. Transplacental passage of acetaminophen in term pregnancy. *American journal of perinatology*, *34*(06), 541-543 (2017).
- 16. Sachdeva, M., Barta, V., Thakkar, J., Sakhiya, V. and Miller, I. Pregnancy outcomes in women on hemodialysis: a national survey. *Clinical Kidney Journal*, **10**(2), 276-281 (2017).
- 17. 17- Wesselink, A. K., Wise, L. A., Rothman, K. J., Hahn, K. A., Mikkelsen, E. M., Mahalingaiah, S. and Hatch, E. E. Caffeine and caffeinated beverage

consumption and fecundability in a preconception cohort. *Reproductive Toxicology*, **62**, 39-45 (2016).

- 18. Sisti, J. S., Hankinson, S. E., Caporaso, N. E., Gu, F., Tamimi, R. M., Rosner, B. and Eliassen, A. H. Caffeine, coffee, and tea intake and urinary estrogens and estrogen metabolites in premenopausal women. *Cancer Epidemiology, Biomarkers & Prevention*, **24**(8), 1174-1183 (2015).
- 19. Tollånes, M. C., Strandberg-Larsen, K., Eichelberger, K. Y., Moster,D., Lie, R. T., Brantsæter, A. L. and Wilcox, A. J. Intake of caffeinated soft drinks before and during pregnancy,but not total caffeine intake, is associated with increased cerebral palsy risk in the norwegian mother and child cohort study. *The Journal of Nutrition*, **146**(9), 1701-1706 (2016).
- 20. Ghasemi, A., Jeddi, S. and Kashfi, K. The laboratory rat: Age and body weight matter. *EXCLI Journal*, **20**, 1431 (2021).
- 21. Auld, E., Rappleye, J. and Morris, P. PISA for Development: How the OECD and World Bank shaped education governance post-
2015 Comparative Education, 55(2), 197-219 2015. *Comparative Education*, **55**(2), (2019).
- 22. Alansari, A. K. A. Impact of Code Red energy drink on the functions and structure of the kidney of Wistar Albino rats: possible therapeutic effects of blueberry ethanolic extract. *J. Saudi Soc. Food Nutr.*, **13**(1), 114-126 (2020).
- 23. Salazar, J. H. Overview of urea and creatinine. *Laboratory Medicine*, **45**(1), e19-e20 (2014).
- 24. Luginbühl, M. and Weinmann, W. Creatinine in urine–a method comparison. *Drug Testing and Analysis*, **9**(10), 1537-1541 (2017).
- 25. Hanna, D. H., Beshay, S. N., El. Shafee, E. and El‐ Rahman, H. A. A. The protective effect of aqueous extract of Stevia rebaudiana against tartrazine toxicity in male Wistar rat. *Cell Biochemistry and Function*, **41**(8), 1462-1476(2023).
- 26. Asdaq, S. M. B., Mannasaheb, B. A., Orfali, R., Shaikh, I. A., Alshehri, A., Alghamdi, A. and Abdalla, F. M. A. Antidiabetic and antioxidant potential of Crocin in high-fat diet plus streptozotocin-induced type-2 diabetic rats. *International Journal of Immunopathology and Pharmacology*, **38**, 03946320231220178 (2024).
- 27. Hanna, D. H. and R. Saad, G. Induction of mitochondria mediated apoptosis in human ovarian cancer cells by folic acid coated tin oxide nanoparticles. *Plos One*, **16**(10), e0258115 (2021).
- 28. El-Atawy, M.A., Alsubaie, M.S., Alazmi, M.L., Hamed, E.A., Hanna, D.H., Ahmed, H.A. and Omar, A.Z. Synthesis, Characterization, and Anticancer Activity of New N,N′-Diarylthiourea Derivative against Breast Cancer Cells. *Molecules*, **28**(17), 6420 (2023).
- 29. Hanna, D. H., Osailan, R. and Ahmed, H. A. Stevia rebaudiana methanolic leaf extract in Egypt: phytochemical analysis, antioxidant, antilipid Peroxidation, antihemolytic, antimetastatic, and

anticancer properties. *Journal of Food Biochemistry*, **2023**(1), 7161091 (2023).

- 30. Hanna, D. H., Aziz, M. M. and Shafee, E. E. Effective‐by‐method for the preparation of folic acid‐ coated TiO2 nanoparticles with high targeting potential for apoptosis induction against bladder cancer cells (T24). *Biotechnology and Applied Biochemistry*, **70**(5), 1597-1615 (2023).
- 31. El-Atawy, M.A. Hanna, D.H., Bashal, A.H., Ahmed, H.A., Alshammari, E.M., Hamed, E.A., Aljohani, A.R. and Omar, A.Z. Synthesis, Characterization, Antioxidant, and Anticancer Activity against Colon Cancer Cells of Some Cinnamaldehyde-Based Chalcone Derivatives. *Biomolecules*, **14**(2), 216 (2024).
- 32. Mansy, W., Alogaiel, D. M., Hanafi, M. and Zakaria, E. Effects of chronic consumption of energy drinks on liver and kidney of experimental rats. *Tropical Journal of Pharmaceutical Research*, **16**(12), 2849- 2856 (2017).
- 33. Alkhedaide, A., Soliman, M. M., Salah-Eldin, A. E., Ismail, T. A., Alshehiri, Z. S. and Attia, H. F. Chronic effects of soft drink consumption on the health state of Wistar rats: A biochemical, genetic and histopathological study. *Molecular Medicine Reports*, **13**(6), 5109-5117 (2016).
- 34. Shimony, M. K., Schliep, K. C., Schisterman, E. F., Ahrens, K. A., Sjaarda, L. A., Rotman, Y. and Mumford, S. L. The relationship between sugarsweetened beverages and liver enzymes among healthy premenopausal women: a prospective cohort study. *European Journal of Nutrition*, **55**, 569-576 (2016)
- 35. Alnuimy, A. A. Morphological Study of the Effects of Caffeine Beverages Can Cause Birth Defects on Swiss White Mice Embryos. *Scientific Journal for Faculty of Science-Sirte University*, **1**(1), 18-23 (2021).
- 36. Mirmiran, P., Gaeini, Z., Bahadoran, Z. and Azizi, F. Elevated serum levels of aminotransferases in relation to unhealthy foods intake: Tehran lipid and glucose study. *BMC Endocrine Disorders*, **19**, 1-8 (2019).
- 37. Khayyat, L. I., Essawy, A. E., Al Rawy, M. M. and Sorour, J. M. Comparative study on the effect of energy drinks on haematopoietic system in Wistar albino rats. *Journal of Environmental Biology*, **35**(5), 883 (2014).
- 38. 38-. Hassan, W., Noreen, H., Castro-Gomes, V., Mohammadzai, I., Batista Teixeira da Rocha, J. and Landeira-Fernandez, J. Association of oxidative stress with psychiatric disorders. *Current Pharmaceutical Design*, **22**(20), 2960-2974 (2016).
- 39. 39- Khwairakpam, A. D., Damayenti, Y. D., Deka, A., Monisha, J., Roy, N. K., Padmavathi, G. and Kunnumakkara, A. B. Acorus calamus: a bio-reserve of medicinal values. *Journal of Basic and Clinical Physiology and Pharmacology*, **29**(2), 107-122 (2018).
- 40. 40- Tan, B. L., Norhaizan, M. E., Liew, W. P. P. and Sulaiman Rahman, H. Antioxidant and oxidative

stress: a mutual interplay in age-related diseases. *Frontiers in Pharmacology*, **9**, 1162 (2018).

- 41. El-Shimi, B. I., Mohareb, R. M., Ahmed, H. H., Abohashem, R. S., Mahmoud, K. F. and Hanna, D. Mechanistic Insights into Bisphenol A-Mediated Male Infertility: Potential Role of Panax Ginseng Extract. *Chemistry and Biodiversity,* e202400480 (2024).
- 42. Sharma, D. and Sangha, G. K. Triazophos induced oxidative stress and histomorphological changes in liver and kidney of female albino rats. *Pesticide Biochemistry and Physiology*, **110**, 71-80 (2014).
- 43. Al-Faze, R., Ahmed, H.A., El-Atawy, M.A., Zagloul, H., Alshammari, E.M., Jaremko, M., Emwas, A.H., Nabil, G.M. and Hanna, D.H. Mitochondrial dysfunction route as a possible biomarker and therapy target for human cancer. *Biomedical Journal*,100714 (2024).
- 44. Valle, M. C., Couto-Pereira, N. S., Lampert, C., Arcego, D. M., Toniazzo, A. P., Limberger, R. P. and Leal, M. B. Energy drinks and their component modulate attention, memory, and antioxidant defences in rats. *European Journal of Nutrition*, **57**, 2501-2511 (2018).
- 45. Stefanello, N., Spanevello, R. M., Passamonti, S., Porciúncula, L., Bonan, C. D., Olabiyi, A. A. and Schetinger, M. R. C. Coffee, caffeine, chlorogenic acid, and the purinergic system. *Food and Chemical Toxicology*, **123**, 298-313 (2019).
- 46. Surai, P. F., Earle-Payne, K. and Kidd, M. T. Taurine as a natural antioxidant: From direct antioxidant effects to protective action in various toxicological models. *Antioxidants*, **10**(12), 1876 (2021).
- 47. Abd El-Twab, S. M., Mohamed, H. M. and Mahmoud, A. M. Taurine and pioglitazone attenuate diabetes-induced testicular damage by abrogation of oxidative stress and up-regulation of the pituitary– gonadal axis. *Canadian Journal of Physiology and Pharmacology*, **94**(6), 651-661(2016).
- 48. Damiani, A. P., Garcez, M. L., Letieli de Abreu, L., Tavares, T. H., Rodrigues Boeck, C. and Moraes de Andrade, V. A reduction in DNA damage in neural tissue and peripheral blood of old mice treated with caffeine. *Journal of Toxicology and Environmental Health, Part A*, **80**(13-15), 621-629 (2017).
- 49. Zheng, Y., Qu, H., Wang, D., Li, S., Zhang, C. and Piao, F. Protection of taurine against arsenic-induced DNA damage of mice kidneys. In *Taurine* **10** (pp. 917-927). Springer Netherlands (2017).
- 50. Ayuob, N. and ElBeshbeishy, R. Impact of an energy drink on the structure of stomach and pancreas of albino rat: can omega-3 provide a protection?. *PloS One*, **11**(2), e0149191 (2016).
- 51. El-ghazouly, D. E. S., El-ghazouly, D. E. and Elghazouly, D. E. Histological and Immunohistochemical study on the Effect of the Energy Drink" Red Bull" on the Renal Cortex of Adult Male Albino Rats and the Possible Protective effect of Nigella Sativa oil. *International Journal of Life Sciences*, **6**(4), 142-152 (2017).

تأثير مشروبات الطاقة على أنسجة الكبد والكلى في فئران ويستار الحوامل

 2 دميانـة ح. حنـا ¹¹، ياسمين م. الحبشي ¹، محمد ع. الدسوقي¹، هبه علي عبد الرحمن²

1 قسم الكيمياء - كلية العلوم - جامعة القاهرة – القاهرة - مصر.

2 قسم علم الحيوان - كلية العلوم - جامعة القاهرة - القاهرة - مصر. المؤلف المراسل: دميانة ح. حنا، قسم الكيمياء، كلية العلوم، جامعة القاهرة، القاهرة، مصر (dhelmy@sci.cu.edu.eg)

الملخص

المشروبات غير الكحولية الشائعة التي يتم استهالكها في جميع أنحاء العالم هي مشروبات الطاقة. إن المحتوى العالي من الكافيين في مشروبات الطاقة يحفز الجهاز العصبي المركزي والقلب. يمكن أن تسبب مشروبات الطاقة التهاب وتلف الكبد وزيادة استخالص الماء والصوديوم. تمت دراسة أنسجة الكبد والكلى لدى فئران ويستار الحوامل بعد االستخدام اليومي لمشروبات الطاقة عن طريق الفم. شملت الدراسة ثالث مجموعات من الفئران الحوامل: تلقت المجموعة الضابطة الماء المقطر، وتلقت المجموعة الثانية جرعة منخفضة من مشروبات الطاقة (5 مل / كجم)، وتلقت المجموعة الثالثة جرعة عالية من مشروبات الطاقة (10 مل / كجم). أظهرت النتائج ارتفاعاً معنوياً (P<0.05) في تركيز المالونديالدهيد (MDA) وانخفاضاً كبيراً في مستويات الإنزيمات المضادة للأكسدة في كلا مجموعتي العالج مقارنة بالمجموعة الضابطة. وعالوة على ذلك، زادت مؤشرات وظائف الكبد والكلى لدى المجموعات المعالجة بشكل ملحوظ. أثارت مشروبات الطاقة تغيرات نسجية مرضية في أنسجة الكبد والكلى. يمكن أن يكون الستهالك مشروبات الطاقة أثناء الحمل تأثير ضار كبير على صحة الفئران الحوامل.

الكلمات المفتاحية: مشروبات الطاقة، الحمل، تلف الكبد، تلف الكلى، فئران ويستار.