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# Toxicological Impact of Energy Drinks on the Heart and Liver Tissues of Nursing Female Rats and their Neonates

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**Abstract**

Research and debate surround the global rise in popularity of energy drinks and the paucity of knowledge on their potentially harmful health effects. The purpose of this study was to assess the effects of energy drink consumption on the histology, biochemistry, and DNA of the heart and liver of nursing female rats and their offspring. Wistar female pregnant rats were divided into three groups of gestation (5th- 21st) after birth: the first group (the control) got distilled water; the low dose group got oral administration of energy drinks (10ml/Kg) and the high dose group got (20ml/ kg). The oxidative damage in heart and liver tissues of lactating female rats and neonates was assessed by measuring the levels of malondialdehyde (MDA) and antioxidants superoxide dismutase (SOD) and glutathione reductase (GR). Also, the levels of ALT and AST were evaluated using spectrophotometric methods in serum of all tested groups. Moreover, the levels of Interleukin 6 (IL-6) in the heart and liver tissue of lactating female rats and neonates were assessed using ELISA technique. The possible DNA degradation in the heart and liver tissue of lactating female rats and neonates was investigated in using comet assay. Finally, the histopathological changes in the heart and liver tissue of lactating female rats and neonates were examined. Compared with the control group, the results demonstrated that energy drinks caused a significant increase in serum levels of ALT, AST, and IL-6 in both the mother and neonate groups compared to the control group. Also, it had a significant increase in MDA level and a significant decrease in antioxidant levels of (GR) and SOD in both energy drinks treated as compared to control groups in heart and liver tissues in lactation females and neonates, respectively. Furthermore, the administration of lactating female rats and neonates with low and high dosages of energy drinks resulted in a significant considerable degradation of DNA. The histological changes revealed a harmful effect on the heart and liver tissues of the treated groups in comparison to the control groups. Energy drink consumption has a detrimental and toxic effect on the liver and cardiac tissues. As a result, lactation female rates transmit this toxic effect to the neonates while nursing. Ladies should exercise caution during feeding

**Keywords** Energy drink, Lactation Female, Neonates, DNA Degradation**.**

## 1. INTRODUCTION

Drinks offer probiotics in a more enjoyable and beverage form compared to many other probiotic supplements. They provide a refreshing way to incorporate probiotics into your diet, which can be more appealing to some people than taking capsules or powders **[1].**

Energy drinks (EDs) consist of a blend of water, sucrose, glucose, sodium citrate, carbon dioxide, taurine (0.4%), caffeine (0.03%), gluconolactone (0.24%), inositol, niacin (8 mg), pantothenic acid (2 mg), vitamin B6 (2 mg), B12 (0.002 mg), caramel, riboflavin, natural and artificial flavoring, and coloring agents. However, excessive consumption of EDs can lead to oxidative stress by producing free radicals, which can affect liver and heart tissues **[2-4].**

Cans containing 250 milliliters of energy beverages (Red Bull GmbH, 5330 Fuschl am See, Austria) are sold in Egypt and are accessible globally. There are 50–550 mg of caffeine in each can of energy drinks from different brands. To boost their energy levels and make up for sleep deprivation, athletes, teenagers, and college students take them in huge quantities. They might lessen mental tiredness, boost physical performance, and elevate mood **[5, 6].** Despite this, it has been documented that consuming large amounts of energy drinks of any kind can cause serious, potentially fatal illnesses **[7, 8].**

Among the negative effects mentioned include hepatitis, cardiac arrest, and arrhythmia **[9–11].** The health implications of these drinks, their popularity, and the lack of adequate research on the safety of energy drinks are all hotly debated topics. Energy drinks are becoming a global phenomenon, thus it's important to thoroughly understand their impacts on various body organs by meticulously examining both the short- and long-term consequences of these drinks **[8].** There are numerous instances of energy drinks having negative health impacts on people. For instance, Red Bull is the most widely consumed ED due to its primary gradient of caffeine and taurine. Approximately three out of four athletes who have been seen using caffeine (1,3,7-trimethylxanthine) for its ergogenic potential report it to be one of the most common legal ergogenic aids used before competition **[11]**.

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Taurine, also known as 2-aminoethanesulfonic acid, is a nonessential amino acid that is widely distributed throughout the human body, especially in the heart, brain, and skeletal muscle **[12].** Less research has been done on taurine's ergogenic potential, though. **[13].**

Previous research showed that the effects of caffeine and taurine congestion on repeat-sprint cycling performance and associated physiological and perceptual responses. In a double-blind, cross-over, repeated measures study, 11 male subjects (age 21 ± 2 years; body mass 80 ± 13 kg), each separated by 24 seconds, one hour after ingesting: caffeine (80 mg) and taurine (1 g), equivalent to the amount observed in popular commercial energy drinks, or placebo (maltodextrin ∼1 g) in a gelatin capsule **[13].** Around 73% of American collegiate athletes **[14]** and 42% of British elite athletes **[12,13]** reported consuming it. Because energy drinks are said to have ergogenic benefits, many of these athletes participate in multiple-sprint sports **[15].**

There are several ways in which caffeine affects the cardiovascular system. Vascular beds vasodilate because of its antagonistic action on adenosine A1, A2A, and A2B receptors. Its vasodilatory effect is blocked by caffeine, which functions as an antagonist. Furthermore, caffeine raises serum adenosine levels by inhibiting adenosine receptors. This is caused by adenosine's systemic actions, which excite the circulatory system's chemoreceptors. This could be the potential mechanism by which EDs cause a sharp rise in blood pressure and heart rate. The competitive inhibition of phosphodiesterase by caffeine is another mechanism by which it acts. This inhibition leads to an increase in cardiac cyclic adenosine, which raises cardiac AMP and has a positive inotropic effect on the myocardium. Additionally, the action of adenosine dilates arteries. Caffeine reduces this effect by inhibiting adenosine receptors. Taurine reduces intracellular calcium and salt excess over time **[16]**.

In an analysis of anecdotal reports of ED-related adverse cardiovascular effects, research paper reported sudden death, abrupt coronary vasospasm, ST-segment elevation myocardial infarction, unmasking of both LQTS and Brugada ECGs, and the emergence of new atrial and ventricular arrhythmias **[17].** A considerable number of these patients lacked any detectable heart irregularities **[18].** A 26-year-old man who smoked and frequently drank 8–10 cans of energy drinks per day was reported to have an abrupt inferior ST-elevation myocardial infarction **[19].**

It was also noted that three young, healthy males who drank a lot of energy drinks experienced abrupt atrial fibrillation with severe symptoms **[20].** The fact that these occurrences can happen to young people who are generally healthy and do not have underlying cardiovascular illness attests to the strength of ED effects on arrhythmogenesis, especially when ingested quickly and in high amounts.

In the US, taurine is safe to include in drinks up to a particular dosage. Fish and beef are examples of foods high in protein that naturally contain taurine. Taurine is used by the human body in cells for several functions. Taurine's role in the synthesis of energy is one example. In addition, taurine balances fluids, salts, and minerals and aids in the body's processing of bile acid. Although taurine alone might not be harmful, the additional components in EDs might. Energy drinks can contain sugar, caffeine, and other substances including extracts from herbs **[21]**.

As a result, it is unclear if these drinks are safe. Energy drinks are typically tolerable for adults without underlying medical issues. However, some people have trouble falling asleep and become dehydrated after drinking these kinds of drinks. Additionally, they may make someone uneasy and uptight.

The goal of the current study is to determine the harmful effects of EDs on the lactating female Wistar rats and their offspring. It also shows how energy drinks can have toxic consequences during feeding. Therefore, the effects of ED administration on the histology, biochemistry, and DNA of the heart and liver of lactating female rats and their offspring was assessed.

## 2. MATERIAL AND METHODS

### 2.1 Drugs (chemicals)

Cans of EDs (Red Bull GmbH, 5330 Fuschl am See, Austria) are sold in Egypt in 250 ml cans. Red Bull GmbH, an Austrian firm, is the creator and owner of the Red Bull energy drink brand. Caffeine is the main constituent.

### 2.2 Study design and experimental animals

Under the ethical approval number CU/I/F/48/22, the current study was conducted in the Faculty of Science's Animal House at Cairo University's Institutional Animal Care and Use Committee (Egypt). Eighteen Wistar female pregnant rats were split into three groups for the study. Each group contained six rats, each weighing between 180 and 220 grams.

The rats were raised in the experimental animal section of the Department of Zoology at the University of Cairo, Faculty of Science. The animals were housed in various sanitary plastic cages of 65x25x15 cm and kept in pristine, well-ventilated rooms with temperatures between 20 and 23 degrees Celsius and humidity levels between 40 and 50%. They had unrestricted access to libitium. All rats were kept in a normal, healthy environment at all times with the strictest attention to hygiene and care. The general conditions and behavior of the animals were noticed. The animals were divided into three main groups the administration began at the 5$^{th}$ of gestation till the 21$^{st}$ days after birth (Lactation time):

**Control group:** It consists of 6 animals who received 10ml/Kg distilled water by oral route from the 5$^{th}$ of gestation till the 21$^{st}$ days after birth.

**Low-dose group:** It consists of 6 animals who received 10ml/Kg ED by oral route from the 5$^{th}$ of gestation till the 21$^{st}$ days after birth.

**High-dose group:** It consists of 6 animals who received 20ml/Kg ED by oral route from the 5th of gestation till the 21st days after birth.

**2.3 Sample collection**

After the last day of oral administration, the rats were all weighed. The animals were denied nourishment for the whole night on the final day of the experiment, and they were then anesthetized by 60 mg/kg sodium pentobarbital (IP). Blood was drawn from each sedated rat using a cardiac puncture, placed in a test tube, and centrifuged for 10 minutes at 3000 rpm to collect sera for biochemical analysis. The rats' abdomens were opened, and their hearts and livers were removed, and perfused with cold saline before being processed for further analysis.

**2.4 Biochemical Experiments**

**2.4.1 Aspartate Aminotransferase (AST) and alanine Aminotransferase (ALT) Measurement**

Colorimetric kinetic analysis was used to determine ALT and AST in each sera sample. The levels of ALT and AST were measured by equipment manufactured by Bio-Diagnostic (El Omraniya, Giza Governorate, Egypt), according to manufacturer instructions in Catalogues No. 10 31 **[22]** and 10 61**[22]**, respectively.

**2.4.2 Interleukin 6 (IL-6) measurement**

Using a rat IL-6 enzyme-linked immunosorbent assay (ELISA) kit from Sunlong Biotech Company (Hangzhou, China) according to manufacturer instructions in Catalogues No. SL0657Mo. The levels of IL-6 in the heart and liver tissue of lactating female rats and neonates were assessed in each treatment group by radioimmunoassay.

**2.4.3 Oxidative Stress Marker Evaluation**

The level of MDA **[23],** and the activities of SOD **[23],** and GR **[23]** were measured in the liver and heart tissue extract using the previously published colorimetric method with bio diagnostic kits (CAT. No. MDA 25 29), (CAT. No. SOD 25 21), and (CAT. No. GSH 25 11), respectively. Researchers state that lipid peroxidation was assessed in the homogenates using the thiobarbituric acid reactive substances (TRABRS) analysis at 532 nm, GR measurement at 405 nm, and SOD measurement at 560 nm **[24].**

**2.5 Comet assay analysis**

The comet assay was performed on the heart and liver tissue of nursing female rats and neonates in all treated groups. It is a popular technique for determining whether a certain cell has DNA damage. Damaged cellular DNA, including strand breaks and fragments, separates from intact DNA under the influence of an electric field, resulting in a comet-tail structure visible under a fluorescence microscope. This approach has already been described **[14,25-27]**. 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 evaluate the photos and estimate the comet parameters.

**2.6 Histopathological study**

The histopathological examination was assessed according to previous studies **[ 28, 29].** The liver and heart tissues were biopsied, and the tissues were immediately preserved in 10% natural buffered formalin. The fixative was replaced 24 hours later. The tissues were then dehydrated using a graded ethanol series. Subsequently, the specimen underwent xylene cleaning, paraffin wax embedding, 5 µm sectioning, hematoxylin and eosin (H&E) staining, and a light microscope examination by a blinded histopathologic at Cairo University's, Faculty of Veterinary Medicine, Egypt **[28,29]**.

**2.7 Statistical analysis**

The statistical analysis was carried out utilizing SPSS software, version 17.00. Every data point was presented as mean±SD. To compare the groups, one-way analysis of variance (ANOVA) and post hoc Tukey with the least significant difference were employed. A significant threshold of $p<0.05$ was applied.

**3. RESULTS**

**3.1 Biochemical assay**

In lactation, female Wistar rats, and neonates, the effects of EDs on the liver function test are summarized in **Table 1**. Oral administration of energy drinks at low (10 ml/kg) and high (20 ml/kg) doses resulted in a significant increase in the concentration level of ALT& AST ($p <0.05$) when compared to control.

**3.2 Oxidative stress markers**

As shown in **Table 1**, oral administration of ED (10ml/Kg) as a low dose and (20ml/Kg) as a high dose in comparison to the control group caused a significant increase in MDA level ($p <0 .05$) in both heart and liver tissues of lactated Wistar rates and neonates groups. Furthermore, the lactated Wistar rates group's heart and liver tissues (low and high dose group) showed a markedly lower level of SOD activity in comparison to the control group. In contrast to the control group, the neonates treated groups (low and high dose groups) showed a substantial drop in liver tissues and an increase in heart tissues in SOD activity. Moreover, the activity of GR was decreased in the lactated Wistar rates treated groups (low and high dose groups) in both heart and liver tissues compared to control group. The nenoates groups (low and high dose groups) showed significant decreased in GR activity compared to control group in heart tissue, but it showed significant increase in GR activity compared to control group in liver tissue.

### 3.3 Serum Interleukin 6 level (IL-6)

As shown in **Table 1**, oral administration of ED low dose group (10mg/Kg) and high doses group (20mg/Kg) caused a significant increase in the level of interleukin 6 levels (IL -6) protein ($p < 0.05$) as compared with the control group in both nursing Wistar rats and neonates.

**Table 1.** Effect of ED on the levels of liver function, oxidative markers and IL-6 inflammatory marker in the in serum, heart, and liver tissues of lactation female and their neonates in the different studied groups.

| **Parameters** | **Control group** | | **Low dose group** | | **High dose group** | |
|---|---|---|---|---|---|---|
| | **Mother** | **Neonates** | **Mother** | **Neonates** | **Mother** | **Neonates** |
| **SALT** (U/L) | 98.33± 3.27 | 91.87±1.45 | $123.17±9.35^{a}$ | $109.5±4.23^{a}$ | $110±10.23^{ab}$ | $123.67±4.18^{ab}$ |
| **SAST** (U/L) | 189± 5.69 | 182.83±13.93 | $214.33±10.31^{a}$ | $248±15.67^{a}$ | $207.33±17.21^{ab}$ | $333.5±10.13^{ab}$ |
| **SIL-6** (pg/ml) | 86.52± 5.03 | 69.3± 4.82 | $114.5± 5.06^{a}$ | $83.75±5.82^{a}$ | $121.6± 3.62^{ab}$ | $95.02± 2.62^{ab}$ |
| **Heart** | | | | | | |
| **MDA** (nmol/g. tissue**)** | 161.8± 13.47 | 50.12±1.63 | $263.443±22.87^{a}$ | $57.07±1.73^{a}$ | $290.178±17.08^{ab}$ | $70.52±1.60^{ab}$ |
| **GR** (mg/g.tissue) | 2.80± 0.270 | 2.91± 0.17 | 2.23±0.293 | $2.00 ± 0.24^{a}$ | $1.925±0.156^{ab}$ | $1.90± 0.30^{ab}$ |
| **SOD** (U/g.tissue) | 77.83± 5.94 | 8.18± 0.39 | $56.82±5.11^{a}$ | $9.41± 0.52^{a}$ | $44.15±7.37^{ab}$ | $9.41±0.71^{a}$ |
| **Liver** | | | | | | |
| **MDA** (nmol/g. tissue**)** | 6.88± 5.92 | 37.28± 2.71 | $33.22±1.16^{a}$ | $53.12±3.28^{a}$ | $36.16±0.792^{ab}$ | $55.31±3.53^{ab}$ |
| **GR** (mg/g.tissue) | 17.02± 1.20 | 2.58± 0.43 | $13.74±0.53^{a}$ | $4.02 ± 0.23^{a}$ | $12.46±0.55^{ab}$ | $5.77± 0.41^{ab}$ |
| **SOD** (U/g.tissue) | 27.16± 0.74 | 49.84± 5.68 | $33.22±1.16^{a}$ | $36.18±3.64^{a}$ | $30.16±0.79^{ab}$ | $31.27±31.27^{ab}$ |

Note: Data are presented as mean ± SD of six values in each group (n = 6).
a: $p<0.05$ compared to the control group and b: $p < 0.05$ compared to the low-dose group.

### 3.4 Comet assay results

The assessment of DNA damage in heart and liver tissues were studied using a comet assay and the comet assay parameters for DNA damage are presented in **Tables 2&3 and Figures 1&2.** When ED (10 ml/Kg) as a low dose and (20ml/Kg) as a high dose were given orally, it significantly increased the olive tail moment (OTM), $p < 0.05$. as compared with the control group in both treated groups of lactated female Wistar rats and neonates.

**Table 2.** Effect of ED on the DNA of the heart lactation female and neonate tissues using comet assay in the different studied groups.

| **Parameters** | %DNA in tail | | Tail moment | | Olive tail moment | |
|---|---|---|---|---|---|---|
| **Groups** | Mother | Neonates | Mother | Neonates | Mother | Neonates |
| Control | 6.41 ± 0.64 | 4.22 ± 0.43 | 0.68 ± 0.06 | 0.33 ± 0.1 | 0.83 ± 0.08 | 0.67 ± 0.16 |
| Low groups (10ml/kg) | $10.01 ± 0.97^{a}$ | $9.99 ± 0.69^{a}$ | $0.89 ± 0.06^{a}$ | $1.17 ± 0.15^{a}$ | $1.38± 0.09^{a}$ | $1.50± 0.13^{a}$ |
| High groups (20ml/Kg) | $16.05 ± 1.46^{ab}$ | $21.81 ± 0.57^{ab}$ | $1.94 ± 0.12^{ab}$ | $1.96 ± 0.42^{ab}$ | $2.18 ± 0.15^{ab}$ | $2.83 ± 0.30^{ab}$ |

Note: Data are presented as mean ± SD of six values in each group (n = 6).
a= $p<0.05$ compared to the control group.
b=$p < 0.05$ compared to the low-dose group.

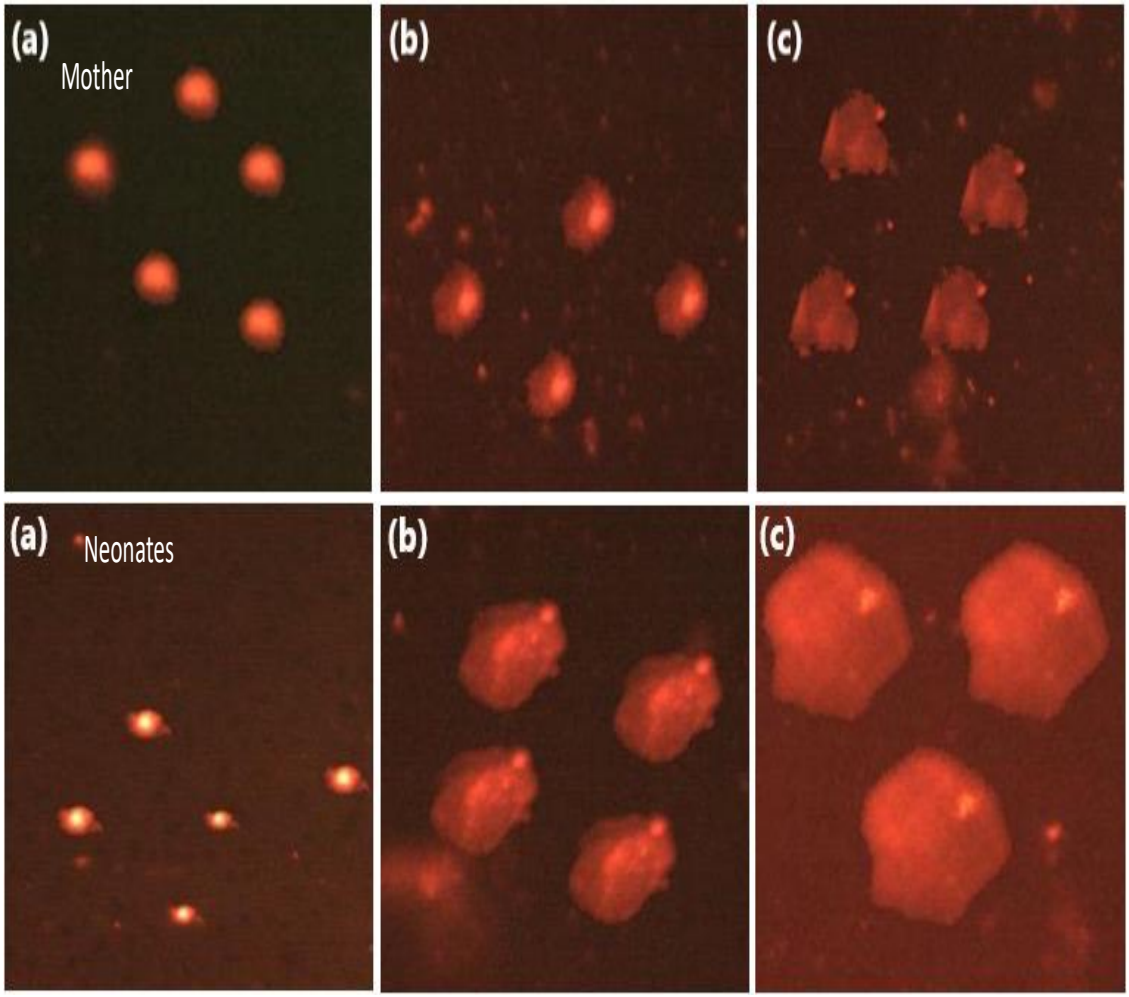


**Figure 1**: Fluorescent microscope pictures for the effect of energy drinks on the nucleus of low dose group (b) and high dose group (c) compared with the nucleus of control group (a) in the Heart mother & neonates tissue.

**Table 3. The Effect of ED on the DNA of the liver nursing female and neonate tissues using Comet Assay in the different studied groups.**

| **Parameters** | %DNA in tail | | Tail moment | | Olive tail moment | |
|---|---|---|---|---|---|---|
| **Groups** | Mother | neonates | Mother | neonates | Mother | neonates |
| Control | 5.14 ± 1.49 | 5.82± 1.27 | 0.63 ± 0.10 | 0.55 ± 0.07 | 0.80 ± 0.25 | 0.69 ± 0.25 |
| Low groups (10ml/Kg) | 9.36 ± 1.36 [a] | 7.68 ± 0.74[a] | 1.17 ± 0.15 [a] | 1.27 ± 0.06 [a] | 1.18 ± 0.16 [a] | 1.58± 0.05 [a] |
| High groups (20ml/Kg) | 14.91 ± 1.0 [ab] | 9.42 ± 0.78[ab] | 1.43 ± 0.31[ab] | 1.32 ± 0.11[ab] | 2.05 ± 0.82[ab] | 1.68 ± 0.05[ab] |

Note: Data are presented as mean ± SD of six values in each group (n = 6).
a= $p<0.05$ compared to the control group.
b=$p < 0.05$ compared to the low-dose group.

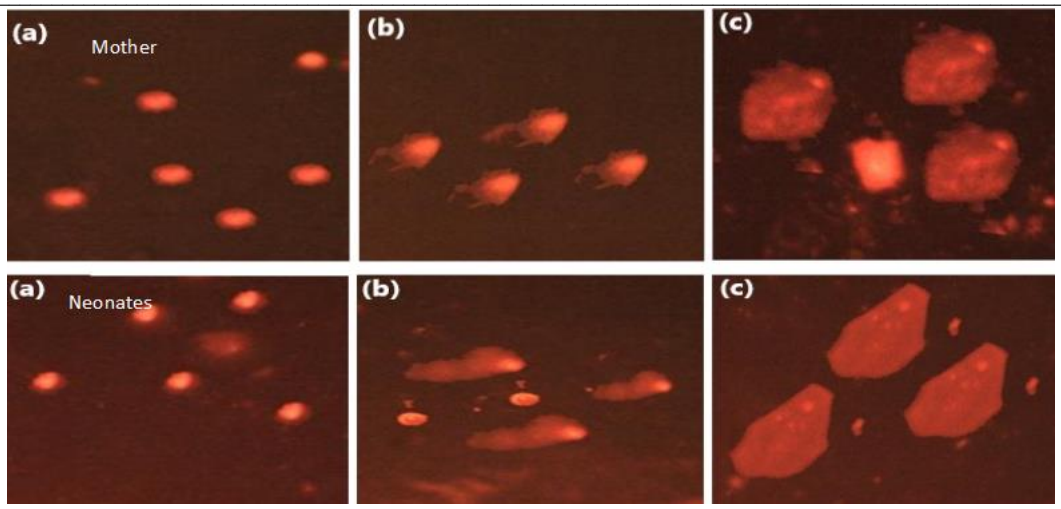


**Figure 2**: Fluorescent microscope pictures for the effect of energy drinks on the nucleus of low dose group (b) and high dose group(c) compared with the nucleus of control group (a) in the Liver of mother & neonates tissue.

### 3.5 Histopathological changes

#### 3.5.1 Effect of energy drinks on nursing female Wistar rats & neonates on the heart tissue

**Control group:**

The histological structure of heart sections of lactated female rats and neonates appeared to be normal without any histopathological changes, cardiac myocytes comprise longitudinal muscle fibers that branched and anastomose, acidophilic sarcoplasm, and oval vesicular nuclei in the center **(Figure 3a)**.

**Low and High-dose treated groups:**

There are areas where myocyte cytolysis and localized degeneration are present. Vacuolation and congestion were both discernible. The low dose group of neonatal heart tissue section's MF striation in the induced group exhibits an altered and uneven appearance. Patches are disturbed in cardiomyocytes without sarcoplasmic striations. Nuclei in decline. Signs of blocked and swollen blood vessels were present. Sarcoplasmic striations are absent from cardiomyocytes, resulting in discontinuities **(Figure 3b)**. The high dose group exhibits an exaggerated MF stiation. In addition to myocyte cytolysis, there are areas of localized degeneration. It was possible to see congestion and vacuolation. Sarcoplasmic striations are absent from cardiomyocytes, resulting in discontinuities. In comparison to the control group, there were more hyperacidophilic cytoplasm, pyrocytic nuclei, and lymphatic infiltration, **Figure 3c.**

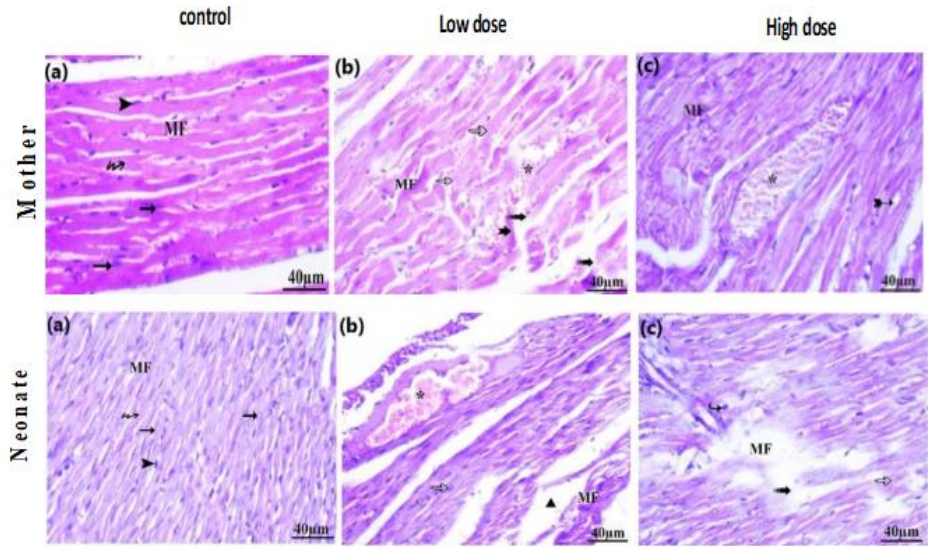


**Figure 3**: Effect of ED on lactating female Wistar rata and neonates in the Heart tissueH&E staining for the morphological characteristic of control group (a), treated low dose group (b) and high dose group (c) with ED.The striation of the heart muscle (MF) is misrepresented. There are regions of localized degeneration and myocyte cytolysis (dotted arrow). Visible were vacuolation (bifid arrow) and congestion (*). Cardiomyocytes lose their sarcoplasmic striations, resulting in areas of discontinuity (triangle). Hyperacidophilic cytoplasm (notch arrow), Cytoplasm that is less stained and has lost its striation (bold arrow).

**3.5.2 Effect of energy drinks on nursing female Wistar rats and neonates on Liver tissues**

**Control group:**

The histological structure of liver sections in the control group appeared to be normal without any histopathological changes. Hepatic cells were found to be coordinated in strands emanating from the central veins, illustrating the traditional hepatic structure. Hepatocytes were polyhedral, with acidophilic cytoplasm and spherical and central nuclei. Also, hepatic sinusoids were discovered to be thin spaces between hepatic cords lined by endothelial cells and a few Kupffer cells. Portal tracts are a branch of the portal vein and a bile duct located at the periphery of the hepatic lobule, as shown in **Figure 4a.**

**Low and High-dose treated groups:**

There is a disruption of ordinary hepatic cells, moderate fatty degenerative changes, and altered lobular configuration, and nuclear degeneration. The polyhedral hepatocytes (H) had spherical and central nuclei and acidophilic cytoplasm. The portal vein (PV) and hepatic central vein (CV) also showed effects in the treated groups, with the overdose group seeing more expansion and blockage than the low-treatment group. Hepatocytes exhibiting cytoplasmic vacuoles, pyknosis, and karyolitic characteristics are distinguished from one another. Necrosis and lymphatic infiltration (LI) of the bile duct (BD) were found, invasion of the BD by lymphocytes **(Figure 4)**. .

The results of the high dose group demonstrate the disarray of normal hepatic cells, hydropic deteriorating (swollen hepatocytes with small shrinkage nuclei) cells that changed lobular shape, and nuclear deterioration in specific locations. These observations were all made in induced liver sections. Along with congestion, an enlargement of the hepatic PV and CV was discovered. It was discovered that LI had entered the portal region. Pyknosis, or degenerative change, was evident in some liver cells **(Figure 4c)**. On the other hand, the second treatment group (low dose group) showed signs of fatty degenerative alterations, disturbance of normal liver cells, lobular organization, and nuclear breakdown in some locations. The hepatic CV had a wavy arrow-shaped distracted wall. In the vicinity of the BD and pv, LI were seen, logged portal vein. Dilated sinusoid (S), hydropic degeneration, and pyknotic nuclei **(Figure 4b)** .

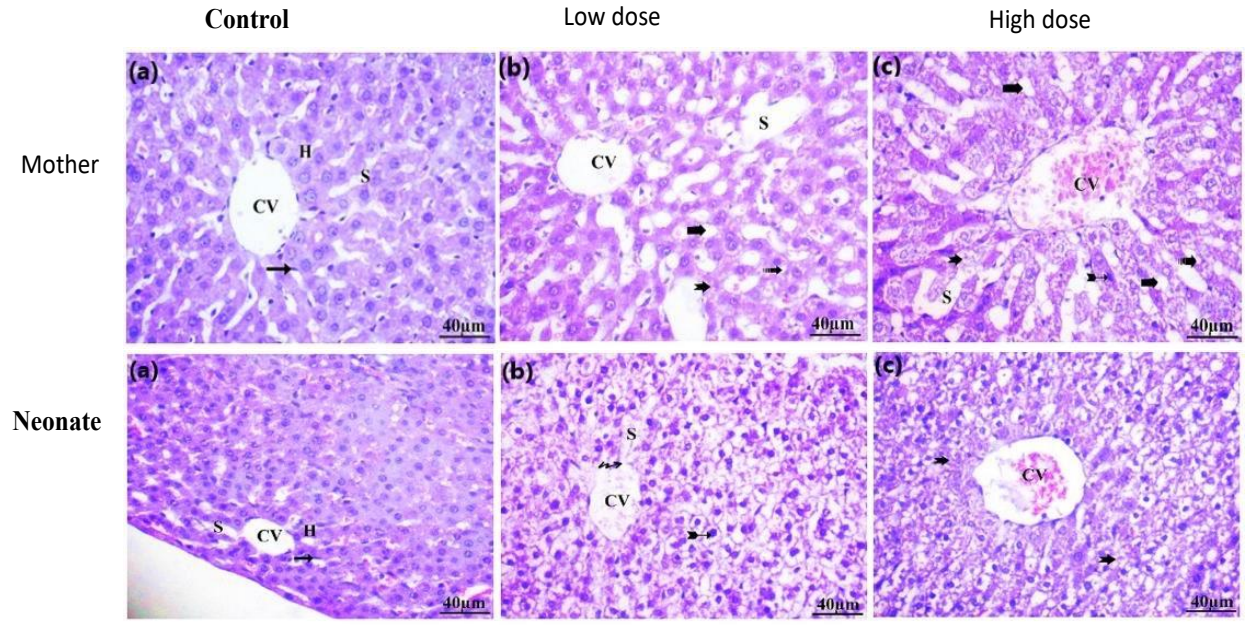


**Figure 4:** Effect of ED on lactating female Wistar rats and neonates in the Liver tissue

H&E staining for the morphological characteristic of control group (a), treated low dose group (b) and high dose group (c) with ED. The hepatic central vein (CV) and portal vein (PV) were both expanded and clogged. Hepatic cells with cytoplasmic vacuoles (dotted arrow), and other hepatocytes with pyknosis (bifid arrow). Lymphatic infiltration (LI) of the bile duct (BD) and necrosis was detected.

**DISCUSSION**

This work aimed to evaluate the consumption effects of EDs in gestation period (5th–20th), focusing on the nursing Wistar Rat and their neonate relationship. The high consumption of EDs is primarily linked to caffeine as a major ingredient in energy drink content.

Long-term use of energy drinks has been connected to histological alterations in the ultrastructure and biochemistry of the heart muscle, as well as oxidative imbalance and liver tissue damage **[30]**, and it has been concluded that it is crucial to therapeutically prevent the associated problems in youth. Previous studies looked into how long-term Red Bull® consumption affected the myocardium's ultrastructure and a few other biochemical markers [**16]**. The stimulatory effects of caffeine and the influence of taurine in tissue allow us to hypothesize that these substances control the effects of caffeine based on the mechanisms of caffeine and taurine in the liver and heart tissue **[31, 32].**

The results of the investigation into the impact of EDs on DNA degradation in all rat groups revealed that the high-dosage group experienced more severe DNA degradation than the low-dose group, suggesting that energy drinks have a toxic effect on both tissues. Comparing the olive tail moment (OTM) values of the heart and liver tissue of nursing moms and newborns in the high and low groups to the control groups, a substantial increase was seen. The size of the fragment and the proportion of single-stranded breaks and alkali labile sites are thought to be closely related to the migration length **[33]**. These findings are consistent with earlier research showing that when maternal aspartame was given to rats during pregnancy, there was a significant increase in the percentage of DNA fragmentation in the offspring and the rats themselves, along with enormous structural and numerical chromosomal abnormalities at doses of about 50 mg/day. These results were associated with the build-up of methanol and formaldehyde adducts, metabolites formed from aspartame, which exhibited cytotoxicity through protein functional alterations and DNA mutations that resulted in cell death or cancer. Moreover, oxidative stress caused by aspartame metabolite might result in nuclear damage, as previously seen in rats that eat up to 1000 mg/kg/day **[34].**

The adverse effect of ED is largely ascribed to its caffeine content and the possible adverse reactions due to the combined enhanced effects of its various components that react as competitive enhancers or effectors to the various tissues. Caffeine is structurally like adenosine and acts as a competitive inhibitor for adenosine receptors **[32]**. Caffeine binds to the receptor in place of adenosine, thus inactivating the inhibitory effect of adenosine binding on dopamine release, leading to further increases in dopamine and greater arousal **[35]**. On the other hand, extracellular adenosine (which is similar in structure to caffeine), functionally contributes to liver homeostasis through modulation of several major metabolic pathways such as glycogenesis, ureagenesis, and lipid synthesis, as well as key tissue functions such as bile secretion and maintenance of vascular tone in resting condition **[24].**

According to that the explanation for the high elevation of ALT and AST in the serum of both lactation rats and their neonates, which may be because of a liver injury or disease. Some types of liver disease cause high ALT, also consumption of ED in both groups may lead to liver damage or liver disease, which is associated with high levels of serum ALT and AST.

This study has demonstrated that oral administration of ED to rats for 4 weeks resulted in varying degrees of liver damage this was evidenced in the ED-induced significant elevation in serum AST and ALT levels. Increases in the serum levels of hepatic enzymes serve as reliable indicators of liver damage by toxic agents. Similar increases have been reported in serum AST and ALT of rats exposed to EDs **[36].** Moreover, values of AST and ALT were elevated in high versus low dose ED indicating more toxicity of high dose ED on the liver and appeared in circulation **[37]** this makes sense as hepatic failure was observed in both treated groups specifically in the high dose group than low dose group in both lactation Wistar rats and their neonates.

Previous studies found no changes in resting heart rate about the effects of caffeine on heart rate **[38],** however, other researchers observed an increase in heart rate as well as in the high-frequency component of heart rate variability **[39]**.The altered change in the heart tissue may be because adenosine interrupts electrical signals in the heart that induce irregular heartbeats **[11]**.Thus the obtained findings of the serum IL-6 values were markedly elevated in both nursing female rats and their neonates in all treated groups when compared to the relative control group.The current findings of the heart function of rats given varying doses of code red EDs were supported by the results of previous studies, which were identical in nature **[40]**. The current investigation also showed that the rats' oxidative stress markers increased after being exposed to ED. The ED-induced substantial reductions in SOD activity in the liver neonate and heart mother groups were indicative of this. Superoxide dismutases can convert two superoxide molecules into a single molecule of hydrogen peroxide (H2O2) and one molecule of water **[41]**.

These crucial antioxidant enzymes collaborate with the non-enzymatic antioxidant system to shield cells from free radical-induced oxidative damage **[42]**. The extremely reactive superoxide anion is neutralized by SOD by turning it into hydrogen peroxide, which is then broken down into water by glutathione peroxidase (GPx) and catalase (CAT). ED-induced elevations in superoxide radicals may overpower the antioxidant enzymes' ability to neutralize them, as seen by the large reductions in tissue homogenate levels of these enzymes, particularly in the rats that received low and high doses of ED.

Earlier research has demonstrated that while low amounts of caffeine did not affect the antioxidant capacity of cells, large quantities of caffeine exposed human cells to the drug creating a pro-oxidant environment in the cells, and increasing protein oxidation. Caffeine has been shown to dramatically raise blood urea nitrogen (BUN) levels, which in turn activates xanthine oxidase. This process then promotes the conversion of xanthine to uric acid, the production of superoxide anion, and $H_2O_2$, where the free radicals are created when $H_2O_2$ and $O_2$ mix [**43**].

Regarding the negative effects of ED as found in this study, many investigators concur. Others claimed that while Red Bull and Power Horse significantly affected liver enzyme activities, they had no discernible effect on liver histopathology. The differences in liver and heart function parameters observed in rats exposed to varying doses of ED were consistent with the lesions visible in the tissue photomicrographs. The lesions that have been detected are most likely a result of ED's harmful consequences. It is conceivable that oxidative stress caused by ED caused tissue damage, which resulted in the lesions. These findings are in line with an earlier study that found signs of hepatotoxicity and changes to the liver's ultrastructure in rats given various ED treatments. In a different study, the damages found in the liver cells were linked to a possible interaction between taurine and another powerful ED component, like caffeine **[20,44]**.

The several components of EDs have the potential to alter the physiology of the cardiovascular system both singly and collectively, which hurts the system. Caffeine, taurine, and carbohydrates are the major components that give these beverages their stimulatory effect. Each of these components affects the cardiovascular system via a distinct method of action. However, earlier research observed a substantial increase in systolic blood pressure (5.2 mmHg) and diastolic blood pressure (6.1 mmHg), as well as increases in heart rate of roughly 3.7 beats per minute and cardiac output, in a randomized cross-over study comparing Red Bull (114 mg of caffeine) against tap water **[17]**. The overall peripheral vascular resistance and microvascular endothelial function remained mostly unchanged.

Caffeine affects the cardiovascular system in several ways. It causes vascular beds to vasodilate by acting as an antagonist of adenosine A1, A2A, and A2B receptors. As an antagonist, caffeine prevents the vasodilatory effect of this substance. Additionally, caffeine raises serum adenosine levels by inhibiting adenosine receptors. This is because adenosine stimulates the chemoreceptors in the circulatory system, which has systemic effects. This could be the mechanism by which EDs cause a sharp rise in blood pressure and heart rate. Another method by which caffeine acts is through competitive inhibition of phosphodiesterase, which raises cardiac cyclic adenosine and, consequently, cardiac cyclic adenosine monophosphate (AMP). The vasodilatory activity of adenosine causes arteries to dilate and has a beneficial inotropic effect on the heart. Caffeine reduces this effect by inhibiting adenosine receptors **[16].**

The liver section of this research paper compares induced liver sections in nursing mothers and their neonates to normal hepatic cells, which were found to be coordinated in strands emanating from the central veins (CV), illustrating the traditional hepatic structure. The obtained findings for the histological alteration of liver tissue in nursing western rats and their neonates were in line with a prior study that found signs of hepatotoxicity and changes in liver ultrastructure in rats given several EDs **[18].** In a different investigation, taurine's possible interaction with another active ED substance, like caffeine, was linked to the lesions found in the liver tissues **[45].** Furthermore, other research paper of lipid droplets, which were linked to degenerative alterations in the hepatocytes **[18].** When compared to their mother, newborns who are breastfed have a milder effect, which could indicate that Energy drink has a greater effect on their mother's oral tissue than feeding does. This was in line with every previous work and might explain how feeding a mother and her newborn affected their energy expenditure at either a low or high dose.

The findings from this research paper indicate that the induced low-dosage group's heart muscle striation (MF) was uneven in appearance and had changed in shape. There are discontinuities (triangles) in cardiomyocytes because they lack sarcoplasmic striations. The turn arrow indicates LI, the curved arrow indicates Pyknotic nuclei, and the notch arrow indicates hyperacidophilic cytoplasm. There were signs of blood vessel enlargement and congestion as well as signs of clogged and swollen blood vessels. However, the MF striation is exaggerated and misinterpreted in the induced high-dose group.

Patients with anatomically defective hearts are perhaps at significantly higher risk. After ingesting three Red Bull ED over the course of three to four hours, a patient with corrected Tetralogy of Fallot experienced non-sustained ventricular tachycardia (VT) and ultimately developed Ventricular fibrillation, as reported by researchers **[46]**. This clarifies how the tetratomic effect of ED on the cardiac tissue in the rats' experiment could result in heart attacks or cardiovascular disease. In our trial, two to three rats perish at the start, some of which receive high doses and others modest doses, which account for the different changes in the heart tissue's rate of genetic exchange in the DNA.

**Conclusion:**

The use of ED during lactation may have detrimental effects on mother rats and their offspring, according to the study's findings, the heart defects of the offspring, the degradation and fragmentation of liver tissue, the elevation of serum IL-6 levels, and increase in the liver enzymes (ALT and AST). It was also determined that EDs have dose-dependent effects, thus lactating women should avoid consuming them during this time to prevent these negative consequences.

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**Conflict of interest**

The authors declare no conflict of interest

**Reference:**

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