Original Article

Toxic Effects of Energy Drink Consumption on Cardiac and Renal Function in Female Wistar Rats

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ABSTRACT

Background: Energy drinks (EDs) are widely used non-alcoholic beverages across the globe. Because energy drinks contain a lot of caffeine, they stimulate the heart and central nervous system.

Objectives: The current study aimed to investigate the possible toxic impacts of the EDs on the heart and kidneys in Wister rats.

Methods: Eighteen healthy female albino rats were randomly assigned into three groups (6 rats per group). Low- and high-dose groups received oral ED at 5 and 10 mL/kg body weight/day, respectively, for 4 weeks, while distilled water was given to the control groups. Uric acid, creatinine, urea, creatine kinase, and creatine kinase-MB were measured using the colorimetric method. Moreover, DNA degradation was measured using a comet assay. Inflammatory markers, including interleukin-6 (IL-6), were measured by enzyme-linked immunosorbent assay (ELISA). Finally, heart and kidney tissues were examined for histopathological alterations.

Results: There were significant increases in creatine kinase, CK-MB, creatinine, uric acid, malondialdehyde (MDA), and interleukin-6 compared with the control group. In contrast, the activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) were considerably decreased in both heart and kidney tissues in both ED-treated groups relative to controls. Additionally, there was a considerable increase in DNA damage in both the low- and high-dose groups compared with the control group. Finally, ED induced histopathological changes in kidney and heart tissues, including pyknosis, inflammation, and injury to cardiac muscle fibers.

Conclusion: Cardiac and renal damage were noticeably produced in rats orally exposed to ED. Therefore, we recommend reducing its use and studying its long-term effects.

Keywords: Antioxidant enzymes, DNA degradation, Energy drinks, Female Wistar rat, Inflammatory markers

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Introduction

Energy drinks (EDs) are non-alcoholic, typically carbonated beverages formulated to provide a surge of energy. The initial emergence of EDs occurred in Europe and Asia around 1960, driven by demand for energy-providing dietary supplements (Reissig et al., 2009). Numerous Saudi studies indicate that over fifty percent of customers were aged between 13 and 35 years, more than half had been consuming it for over a year, and over 40% reported drinking more than three cans each week. The centers for disease control and prevention revealed that high school kids use EDs at a rate nearly equivalent to their soda consumption (Elsoadaa et al., 2016). The prevalence of ED intake may exceed the projected figures in this self-reporting poll; such polls typically exhibit a significant likelihood of underreporting (Deliens et al., 2015).

EDs are promoted as stimulants, with compelling claims that suggest strength, power, speed, and sensuality, often accompanied by suitable background music (e.g. Power Horse, Red Bull, Full Throttle, Dare Devil, Cocaine, etc.). EDs are predominantly targeted at youth, including students engaging in late-night study sessions, long-distance transportation, and social gatherings (Malinauskas et al., 2007).

The components of EDs typically include caffeine, amino acids (taurine, creatine, and carnitine), plant stimulants (yerba mate, ephedrine, and guarana), simple sugars (glucose and fructose), herbs (ginseng and ginkgo biloba), naturally occurring glucose metabolites (inositol, glucuronolactone, and maltodextrin) (Tahmassebi & Banihani, 2020), and B complex vitamins. The majority of these substances function as stimulants and are excluded from the Food and Drug Administration (FDA) regulatory list in the United States due to the extensive variety of components present in energy drink constituents (Tanne., 2012). Consequently, it is anticipated that the adverse effects will be more pronounced than those associated with beverages containing solely caffeine. The caffeine concentration in EDs ranges from 50 to 505 mg per can, exceeding the caffeine amount in a standard can of Coke (34 mg) (Burrows et al., 2013) An average energy drink may contain as much as 300 mg of caffeine from both added caffeine and sources, like guarana. Guarana's caffeine content (40–80 mg per gram of extract) is not consistently indicated on packaging. Significant alterations in renal function have been described in rats that ingested EDs, highlighting the detrimental side effects of these substances (Khayyat et al., 2013). Additional studies (Ugwuja, 2014) indicate that ED consumption, whether alone or in conjunction with alcohol, is linked to substantial changes in total white blood cell count, plasma calcium, potassium, triglycerides, as well as liver and kidney functioning.

The physiological effects of the active components of EDs and the noted detrimental effects are likely associated with their formulations (Seifert et al., 2011). Due to the extensive consumption of EDs among individuals, it is crucial to examine their detrimental impacts on health and explore their potential adverse effects. The objective of the present experimental study was to elucidate the functions and histological changes in the kidneys and hearts of healthy female Wistar albino rats following the administration of low and high dosages of an energy drink (5 and 10 mL/kg body weight) for a duration of four weeks.

Materials and Methods

Chemical

The energy drink brand name used in the present study was "Red Bull." It was obtained from a local market in Cairo, Egypt, along with all laboratory reagents and fine chemicals from Bio Diagnostics (Doki, Giza, Egypt). All other materials used as reagents or chemicals were of a quality suitable for commercial sale.

Study design and animals

Eighteen female Wistar rats were divided into three groups. Each group consisted of six rats (n=6), weighing between 180 and 220 g. The animals were housed in sanitary plastic cages (65×25×15 cm) and maintained in clean, well-ventilated rooms with temperatures between 20 and 23 ˚C and humidity levels between 40 and 50%. They had ad libitum access to food and water. The rats were maintained in a standard, healthy environment with strict adherence to hygiene and animal care protocols. Animal behavior and health status were monitored regularly.

Grouping:

Control group: this group received distilled water orally for four weeks.

Low-dose group: this group received 5 mL/kg body weight EDs by oral gavage for four weeks.

High-dose group: this group received 10 mL/kg body weight EDs or four weeks.

Sample collection

Sacrifice of the rats was performed by decapitation, and cardiac puncture was used to collect blood from each rat; this blood was allowed to clot. The clotted blood was centrifuged at 3000 rpm for 10 minutes to obtain serum. Each rat had its abdominal wall incised along the mid-ventral line to access the abdominal cavity. Subsequently, after removing the heart and kidney tissues, they were cleaned with saline solution and separated into three sections. The first part was stored at -20 ˚C for biochemical study, the second part was kept for histological examination in 10% formalin, and the third part was placed in phosphate-buffered saline to be prepared for comet assay analysis.

Biochemical analysis

The serum was used to assess various parameters, including kidney function (urea, creatinine, and uric acid) and cardiac/muscle injury markers (total creatine kinase (CK) and CK-MB).

The urea and creatinine levels in all serum samples were determined using the Urease-Berthelot method (Richards & Smith, 2013) and colorimetric kinetic method (Hanna et al., 2023), 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. In addition, the spectrum kits (CAT. NO. CK 238 001 and CAT. No. CKMB 239 000) were employed according to the protocols to quantify the levels of CK and CKMB utilizing a colorimetric method.

Interleukin 6 (IL-6) measurement

A rat IL-6 enzyme-linked immunosorbent assay (ELISA) kit from Sun Long Biotech Company (Hangzhou, China) was used according to manufacturer's instructions in Catalogue No. SL0657Mo. The levels of IL-6 in the heart and kidney tissue of female rats were assessed by radioimmunoassay.

Analysis of oxidative stress markers

The activities of catalase (CAT) (Salazar, 2014), malondialdehyde (MDA) (Hanna et al., 2023), superoxide dismutase (SOD) (Hanna et al., 2023), and reduced glutathione (GSH) were assessed in tissue extract utilizing a colorimetric approach with biodiagnostic kits (CAT. No. MDA 25 17, CAT. No. MDA 25 29, CAT. No. SOD 25 21, and CAT. No. GSH 25 11, respectively).

Comet assay

Single-cell gel electrophoresis (SCGE), also known as the comet assay, was employed to determine DNA damage in heart and kidney tissues (El-Rahman et al., 2025; Hassan et al., 2023; Mustafa et al., 2025; Ismail et al., 2025). A computerized image analysis system captured 100 random comets on each slide. The system then used TriTek Comet ScoreTM software (TriTek Corp.) to analyze the images and calculate the comet parameters. Tail moments and tail DNA are common measures used to assess DNA damage. Regarding DNA mobility and presence in the tail, the olive tail moment (OTM) parameter is considered the most effective measure of DNA damage. It is calculated using Equation 1 (El-Atawy et al., 2023; El-Atawy et al., 2024; Hanna et al., 2024; Sayed et al., 2024).

1. $OTM = Tail\ moment \times Tail/100$

Histopathological examination

The histopathological examination was assessed according to previous studies (Abo Alhamd et al., 2025; Ahmed et al., 2024; Moussa, et al., 2025). Kidney and heart tissue samples were collected during necropsy and immediately preserved in 10% neutral buffered formalin. After 24 hours, the fixative was replaced. Tissue dehydration was performed using a graded ethanol series. Subsequently, the specimens underwent xylene clearing, paraffin wax embedding, 5 µm sectioning, hematoxylin and eosin (H&E) staining, and light microscopic examination by a blinded histopathologist at Cairo University's Faculty of Veterinary Medicine, Egypt.

Statistical analysis

Statistical analysis was done using SPSS software, version 17. Data were presented as Mean±SD. To compare groups, one-way analysis of variance (ANOVA) followed by post hoc Tukey's test were employed. A significance threshold of $P<0.05$ was applied.

Results

Kidney and heart weights

Table 1 shows the effects of the low and high dosages (5 and 10 mL/kg body weight) of EDs consumed for four weeks on kidney and heart weights. The weights of kidney and heart in the ED-treated groups did not noticeably differ from one another

Table 1. Effect of energy drink on kidneys and heart weight in female rats

Parameters	Control Group (n=6)	Low-dose Group (5 mL/kg Body Weight) (n=6)	High-dose Group (10 mL/kg Body Weight) (n=6)
Kidney weight (mg)	108±9.38	110±10.49	111±6.44
Heart weight (g)	63±3.34	63.8±2.92	64.3±3.72

Kidney function parameters

After ED intake, the creatinine and uric acid levels in both treatment groups increased significantly in comparison to the control group, but there was no significant difference in the urea level after ED administration. Additionally, after administering EDs at a high dosage, the creatinine level rose significantly when compared with the low-dosage group, but there was no significant change in uric acid levels between the low- and high-dosage ED-treated groups (Table 2).

Heart function biomarkers

The administration of EDs caused a significant rise in the levels of CK and CK-MB in a dose-dependent manner compared with the control group ($P<0.05$), as illustrated in Table 3.

IL-6 and oxidative status biomarkers

Table 4 shows that only administration of EDs at the high dosage produced a significant change in IL-6 in kidney and heart tissues compared with the control group ($P<0.05$). Also, in kidney and heart tissues, ED intake induced a significant decrease in SOD, GSH, and CAT levels and an elevation in MDA levels in the treated groups compared with the control group ($P<0.05$).

Comet assay result

Table 5 shows that after ED intake, the %DNA in the tail, tail moment, and OTM levels of renal tissue were significantly increased compared with the control group. Significant differences were observed in both ED-treated groups for tail moment and OTM in renal tissue (Figure 1). In heart tissue, the %DNA in tail and OTM levels were significantly increased compared with the control group. There was also a non-significant change in tail moment after ED administration. Moreover, no significant differences were detected between the two treated groups (Figure 2).

Histopathologic investigations

Kidney tissues

The histological investigation of kidney sections from the rats is demonstrated in Figure 3. In the control group, renal corpuscles, Bowman's capsules, glomerular capillaries, and the urinary space fill the renal cortex. The proximal convoluted tubules have a small lumen bordered by cuboidal cells with spherical nuclei. The distal convoluted tubules have a wide lumen surrounded by simple cubical cells with spherical nuclei in the center or at the tip of the tubule walls. In the low-dose group, there were variations in glomerular dimensions and shapes. Total or partial damage to cell nuclei was observed in the proximal and distal convoluted tubules, indicating disintegration.

Furthermore, nuclei collapsed. There was infiltration of inflammatory cells (lymphocytes), and congestion was evident in the renal tissue. Moreover, the high-dose group showed glomeruli that were vacuolated, congested, and swollen. Other glomeruli exhibited vacuolation.

Table 2. Effect of energy drink on kidney function in female rats

Parameters	Mean±SD		
	Control Group (n=6)	Low-dose Group (5 mL/kg Body Weight) (n=6)	High-dose Group (10 mL/kg Body Weight) (n=6)
Creatinine (mg/dL)	0.57±0.04	0.66±0112[a]	0.72±0.07[ab]
Uric acid (mg/dL)	3.1±0.91	5.48±0.4[a]	6.4±0.3[a]
Urea (mg/dL)	23.5±4.2	23.02±3.39	21.4±3.34

[a]$P<0.05$ compared to the control group, [b]$P<0.05$ compared to the low-dose group.

Table 3. Effect of EDs on heart function in female rats

Parameters	Mean±SD		
	Control Group (n=6)	Low-dose Group (5 mL/kg Body Weight) (n=6)	High-dose Group (10 mL/kg Body Weight) (n=6)
Total creatine kinase (U/L)	1680.01±95.4	1907.16±79.11[a]	2260.4±131.4[ab]
Creatine kinase –MB (U/L)	108.03±5.59	146.7±20.89[a]	199.8±24.76[ab]

[a]P<0.05 compared to to the control group, [b]P<0.05 compared to the low-dose group.

Desquamation and disruption of the renal tubule epithelial linings were observed. In addition, nuclei appeared condensed. There was infiltration of inflammatory cells (lymphocytes), and considerable congestion. Within the lumens of tubular segments, cellular remnants and exfoliated epithelial cells were seen.

Heart tissues

The histological investigation of sections of the heart from the rats is illustrated in Figure 4. In the control group, cardiac myocytes showed branching and anastomosing longitudinal muscle fibers, acidophilic sarcoplasm, and oval, vesicular nuclei in the center. Interstitial cells with larger nuclei were present.

The low-dose group showed distortion of the striations of the heart muscle. The gaps between cardiomyocytes enlarged significantly. Some cardiomyocytes lacked sarcoplasmic striations, resulting in discontinuous regions. Also, in the high-dose group, the striations of the heart muscle were distorted. There was evidence of dilated and congested blood vessels, as well as cellular infiltration indicative of inflammation. Myocyte cytolysis, vacuolation, and hemorrhage were observed.

Discussion

The increased consumption of EDs in recent years has led to a rise in research studies demonstrating their toxic effects on various organs. The present study examined the effects of daily oral administration of 5 mL/kg/day of energy drink as a low dose and 10 mL/kg/day as a high dose for 28 days on kidney and heart tissues in female Wistar rats. The study showed changes in biochemical markers, DNA integrity, and tissue histopathology. EDs compared with a cup of coffee, contain a much higher concentration of caffeine; therefore, their effects are more pronounced. Sugar is also an ingredient in EDs and has harmful effects. During the 30 days of exposure to the ED, no signs of systematic toxicity were observed, and there were no changes in behavior in the treated groups.

Table 4. Effects of energy drink on IL-6. and oxidative stress biomarkers in female rats

Tissues	Parameters	Control Group (n=6)	Low-dose Group (5 mL/kg Body Weight) (n=6)	High-dose group (10 mL/kg Body Weight) (n=6)
	IL-6	76.75±2.91	90.9±1.61	104.7±6.9[a]
Kidney	SOD (U/g tissue)	3.0317±0.2778	2.66±0.163[a]	0.72±0.07[ab]
	GSH (mg/g tissue)	3.1±0.91	5.48±0.47[a]	6.4±0.3[ab]
	CAT (U/g tissue)	3.0317±0.277	2.66±0.163[a]	1.95±0.187[ab]
	MDA (nmol/g tissue)	67.08±4.017	76.2±2.04[a]	84.36±2.34[ab]
Heart	SOD (U/g tissue)	57.43±4.16	50.36±3.9[a]	42.533±3.76[ab]
	GSH (mg/g tissue)	90.38±2.166	83.96±2.455[a]	70.433±1.496[ab]
	CAT (U/g tissue)	2.8±0.47	2.3±0.147[a]	1.95±0.187[a]
	MDA (nmol/g tissue)	30.4±2.187	38.38±2.58[a]	46.15±2.32[ab]

[a]P<0.05 compared to the control group, [b]P<0.05 compared to low dose group.

Table 5. Effect of energy drink on the comet assay parameters for DNA damage of kidney and heart in female rats

Tissues	Parameters	Control Group	Low-dose Group (5 mL/kg Body Weight)	High-dose Group (10 mL/kg Body Weight)
Kidney	%DNA in tail	5.92±0.47	8.76±1.13[a]	9.39±0.49[a]
	Tail moment	0.44±0.03	0.61±0.04[ab]	0.86±0.02[ab]
	Olive tail moment	0.81±0.05	1.37±0.05[ab]	1.54±0.03[ab]
Heart	%DNA in tail	4.95±0.1	8.08±1.33[a]	8.74±1.02[a]
	Tail moment	0.48±0.04	0.54±0.1	0.57±0.07
	Olive tail moment	0.72±0.04	1.1.26±0.22[a]	1.23±0.11[a]

[a]P<0.05 compared to the control group, [b]P<0.05 compared to the low-dose group.

Effect of energy drink on kidney function

The current investigation revealed a significant surge in blood uric acid and creatinine levels in both treated groups relative to the control group. An elevation in blood levels of creatinine and uric acid is typically linked to compromised renal function. These findings are consistent with those of Khayyat et al. (2014), who showed that EDs caused increases in serum creatinine and uric acid levels. The researchers proposed that caffeine elevated creatinine and uric acid levels by inhibiting A2A adenosine receptors, leading to interstitial inflammation, increased proteinuria, and harmful alterations in renal function and structure. This was corroborated by another study involving male adult albino rats that received varying doses of EDs (0.4, 1.1, and 2.2 mL/100 g body weight/day) over a 12-week period. The current study found no elevation in serum urea levels in either the low-dose group or the high-dose group when compared to the control group, corroborating findings from other researchers who indicated no correlation between caffeine and serum urea concentration in rats. In their investigation, Cheul et al. (1997). utilized female Sprague-Dawley rats' livers. The animals received varying dosages of caffeine over a period of 30 days.

Nonetheless, some research on EDs has reported data that contradict these outcomes. Consumption of EDs has been linked to elevated plasma total protein and reduced levels of creatinine, albumin, and uric acid (Ebuehi et al., 2011). In other researchers' studies, no noticeable correlation was found between caffeine intake and the serum levels of urea and creatinine in rats (Phillips et al., 2014). These variations in the effects of EDs may be attributed to differences in the contents of these energy beverages. On the other hand, it is known that one of the most important components of EDs is sugar, and sugar abundance is related to the generation of reactive oxygen species (ROS) (Haidara et al., 2009). ROS can accelerate kidney disease progression (Daenen et al., 2011). This aligns with histological alterations in kidney tissue, characterized by variations in glomerular dimensions and morphology,

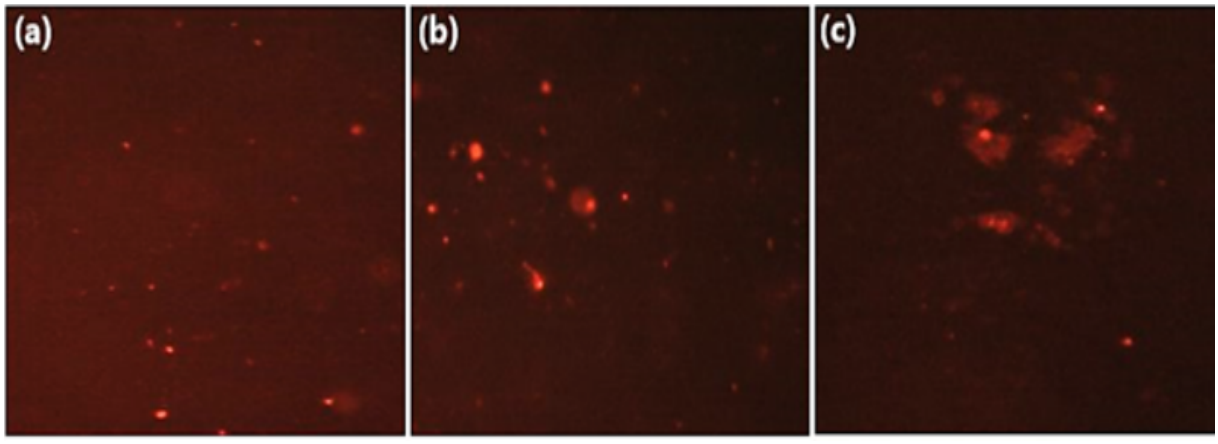

Figure 1. Effect of energy drink on DNA integrity in kidney tissue

a) Group 1 (control group), b) Low-dose group (5 mL/kg body weight), c) High-dose group (10 mL/kg body weight)

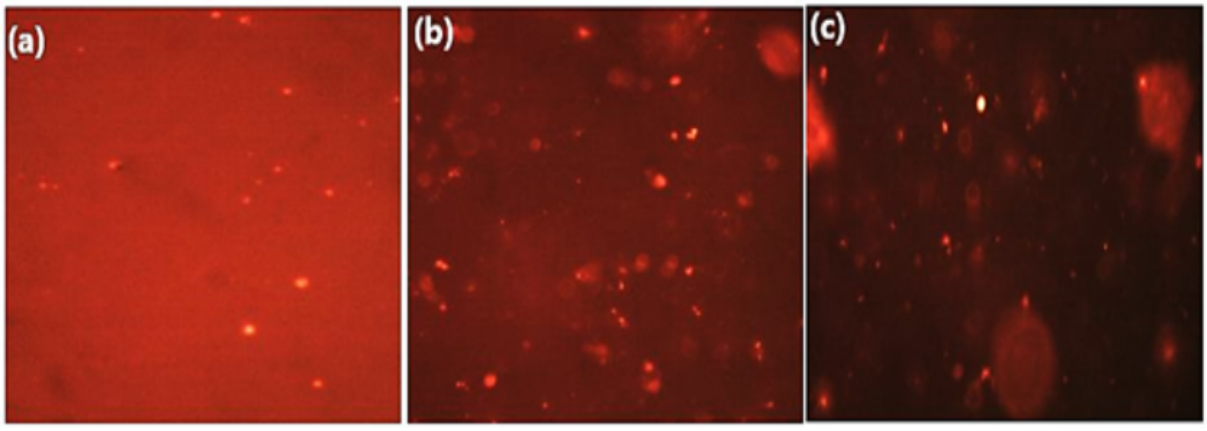

Figure 2. Effect of energy drink on DNA integrity in heart tissue

a) Group 1 (control group), b) Low-dose group (5 mL/kg body weight), c) High-dose group (10 mL/kg body weight)

with specific glomeruli exhibiting size reduction due to atrophied glomerular tufts. Infiltration of inflammatory cells occurs; EDs are recognized for inducing oxidative stress owing to their elevated levels of caffeine and sugar. Oxidative stress can induce inflammation when the body reacts to the harm inflicted by free radicals.

The present findings align with those of other experimental investigations, which indicated that modest doses of EDs cause mild to moderate kidney impairment, whereas large dosages lead to severe damage. Furthermore, instances of renal damage in humans have been documented subsequent to the use of these beverages (Tice et al., 2000). Consequently, it is suggested that chronic intake of EDs is nephrotoxic, with toxicity being dose-dependent.

Effect of energy drink on inflammatory markers

The current investigation demonstrated a notable elevation in IL-6 levels in both treated groups relative to the control group; IL-6 is a protein synthesized by multiple cell types. It assists in modulating immunological responses. IL-6 levels may increase due to inflammation, infection, and cardiovascular diseases. This aligns with other studies indicating that the oral administration of both low and high doses of the energy drink "Code Red" over an 8-week period results in structural changes in rat renal tissue, particularly at high doses, which may significantly contribute to renal damage. Elevated inflammatory marker IL6 (Alansari, 2020). Moreover, EDs are characterized by elevated sugar content, and additional studies have indicated that a diet rich in fructose alters microbiota composition and functionality, which has

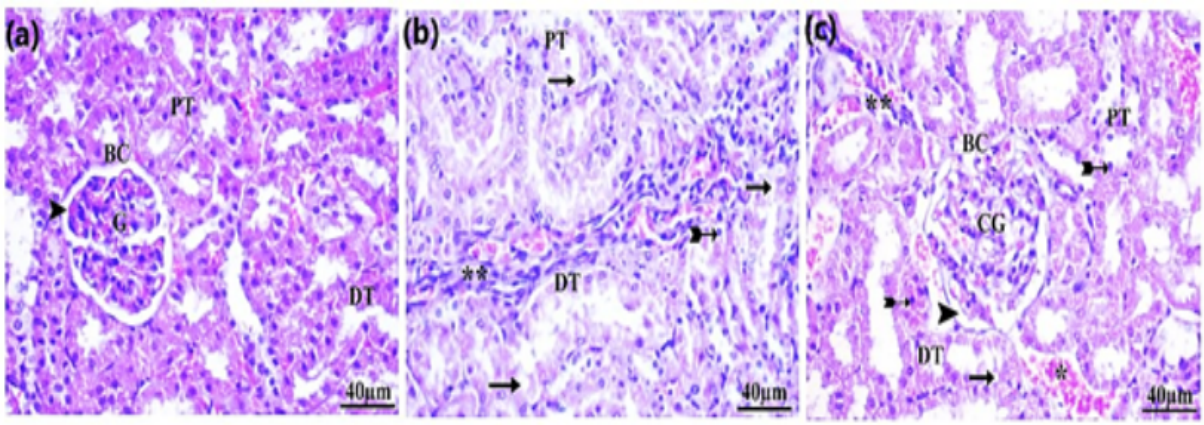

Figure 3. The histological investigation of kidney sections (×600 magnification)

a) Control group: glomerular capillaries (G), Bowman's capsules (BC), proximal convoluted tubules (PT), distal convoluted tubules (DT), b and c) Low-dose group and high-dose group: total or partial cell nuclei damage was noticed in the proximal and distal convoluted tubules, indicating disintegration (arrow), pyknotic nuclei (bifid arrow), invasion of inflammatory cells (**), intra-tubular congestion (*), and congested and vacuolated glomeruli (CG)

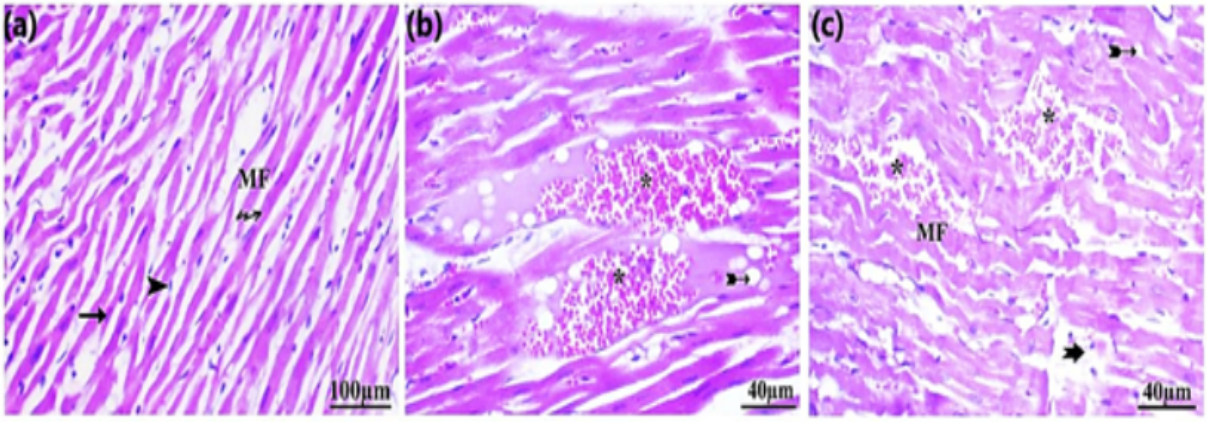

Figure 4. The histological examination of heart sections (×600 magnification)

a) Control group: cardiac myocytes with muscle fibers (MF), acidophilic sarcoplasm (wavy arrow), oval vesicular nuclei (arrow), interstitial cells with larger nuclei (arrowhead), b and c) Low-dose group and high-dose group: striation of the heart muscle is distorted in the induced group, vacuolation (bifid arrow) and hemorrhage (*), cardiomyocytes that lack sarcoplasmic striations, resulting in discontinuous regions and myocyte cytolysis (notched arrow), striation of the heart muscle (MF) is misrepresented

been associated with inflammatory responses. Excessive consumption of dietary sugar has been demonstrated to promote metabolic problems and elevate inflammatory cytokines in many tissues (Ma et al., 2022). Furthermore, this aligns with recent studies reporting that fructose markedly elevated plasma proinflammatory markers, specifically IL-6. In an investigation (Wang et al., 2020), male Sprague-Dawley rats weighing approximately 220 g were utilized. The rats underwent fructose treatment for a duration of 20 weeks.

Effect of energy drink on heart function

CK and CK-MB are biochemical markers related to the heart. CK enters the bloodstream when muscle tissue is damaged and ruptured, releasing its contents into the circulatory system. The current experimental study revealed a substantial elevation in serum CK, indicating muscle cell death. Consistent with earlier studies, it has been observed that EDs may induce hypokalemia due to their diuretic properties and can also elevate creatine kinase levels and lead to renal impairment (Chiang et al., 2022).

The present investigation demonstrated a substantial elevation in the Ck-MB level. CK-MB is a specialized enzyme called myocardial creatine kinase-bound, predominantly found in the heart. Elevated CK-MB values indicate cardiac issues, such as myocardial infarction and myocarditis. The findings of the current study indicated an elevation in CK-MB levels, which contradicts prior research demonstrating a notable reduction in CK-MB levels across all energy drink groups relative to the control group (Backer et al., 2014). Previous research indicated a reduction in heart weight in both low- and high-dose groups; however, a recent study revealed no changes in heart weight. This may explain the disparate outcomes observed between the two investigations. It is already established that excessive sugar generates more free radicals and ROS, which may impact cardiac health. Other evidence indicates the significance of mitochondrial ROS in aging-related cardiac dysfunction and proposes that targeting mitochondrial ROS could serve as an effective therapeutic strategy to safeguard the elderly heart from ischemia-reperfusion (IR) injury (Escobales et al., 2014). This aligns with the histological alterations observed in the heart tissue of both treated groups, where the striation of the cardiac muscle is twisted. The components of EDs, including elevated concentrations of caffeine, sugar, and various additives, may have harmful effects on cardiac muscle. These impacts may disturb normal cellular architecture, leading to misplaced or injured muscle fibers. EDs can elevate oxidative stress, resulting in the formation of ROS that harm cellular components, including myocyte membranes. This oxidative damage may lead to cytolysis.

Previous research indicates that consuming EDs results in histological alterations of cardiomyocytes. Moreover, the ED components induce heightened death of cardiomyocytes and contribute to cardiovascular diseases (Ibero-Baraibar et al., 2015).

Effect of energy drink on oxidative status biomarkers

The findings of the current investigation indicated that both low and high dosages of EDs significantly reduced the activities of SOD, CAT, and GSH, attributable to heightened oxidative stress. The findings align with previous studies (Mansy et al., 2017), indicating a reduction in their activities; this research utilized male adult albino rats administered with EDs at varying doses (0.4, 1.1, and 2.2 mL/100 g body weight/day) over a duration of 12 weeks. These enzymes are crucial antioxidants that collaborate with the non-enzymatic antioxidant system to safeguard cells against damage caused by free radicals (Abdollahi et al., 2004).

Antioxidant enzymes serve as the primary defense mechanism against oxidative stress-induced cellular damage. SOD neutralizes the highly reactive superoxide anion by transforming it into hydrogen peroxide, which is subsequently reduced to water by CAT (Sharma & Sangha, 2014). A reduction in the activity of this enzyme exacerbates the deleterious effects of reactive superoxide anion. In this investigation, MDA showed a substantial increase. MDA is derived from the breakdown of polyunsaturated lipids by ROS. It has been documented to manifest in animal tissues, particularly under antioxidant deficit (Dzoyem et al., 2014).

MDA is a terminal result of the peroxidation of polyunsaturated fatty acids within cells. An elevation in free radicals leads to excessive synthesis of MDA (Reis et al., 2017). These findings are consistent with recent research indicating increased MDA levels in rats subjected to EDs. This study utilized 36 male Sprague-Dawley rats, each weighing between 200 and 250 g, administered various dosages of EDs (3.5 g ED/kg/d, 7 g ED/kg/d, 1 g ethanol/kg/d +3.5 g ED, 1 g ethanol/kg/d +7 g ED/kg/d). All administrations were conducted via oral gavage for a duration of 14 days.

Effect of energy drink on DNA integrity

The comet assay was employed to identify the potential DNA damage following specific therapies. The migration of DNA from fixed nuclear chromatin identifies DNA strand breaks and alkali-labile sites. The present study demonstrated a notable increase in the percentage of DNA in the tail, tail moment, and OTM in both treated groups relative to the control group.

The current findings indicate that the components of EDs induce cytotoxicity or chromosomal damage in the alkaline comet assay. The alkaline comet assay is highly sensitive for rapidly detecting DNA damage in individual cells, facilitating the assessment of DNA damage and repair at the single-cell level. DNA migration depends on the size and extent of DNA fragments. The DNA in the injured cells demonstrates increased migration away from the nucleus, resulting in a comet-like appearance due to the action of a specific chemical (McKelvey et al., 1992). Furthermore, this aligns with findings from other researchers who indicated the presence of DNA damage (Khalaf, 2023).

The study demonstrated a reduction in antioxidants, resulting in an elevation in DNA oxidation and subsequent DNA damage. Research indicates that eating disorders may contribute to an elevation in free radicals and ROS. ROS are acknowledged as mediators of DNA damage. ROS have been documented to directly induce various forms of DNA destruction by oxidizing nucleoside bases (Salehi et al., 2018). Additional studies indicate that the effect of ROS on the DNA damage response is complex and variable, with substantial evidence suggesting that ROS dysregulation contributes to cancer pathogenesis (Srinivas et al., 2019). This underscores the perilous nature of ROS and its effects on DNA integrity.

Conclusion

Both low and high doses of oral EDs administered to female rats can result in significant pathological lesions in the heart and kidneys, potentially impairing their overall function. EDs may induce negative side effects, including significant reductions in antioxidant enzyme levels, increased serum IL-6 levels, and increased indices of renal and cardiac function. The findings underscore the necessity for a comprehensive evaluation of the safety of EDs for individuals. It was also established that EDs exhibit dose-dependent effects. The conclusions derived from this research will be applicable to larger organisms, other animal models, and humans.

Limitation

Additional work is required to understand the fundamental mechanisms and the reversibility of these effects. Investigations are needed to examine the prolonged consequences of habitual energy drink intake. Additional studies are required to evaluate the energy drink's impact on human health.

Ethical Considerations

Compliance with ethical guidelines

The current study was approved by the Animal Care and Use Committee, Faculty of Science, Cairo University's, Cairo, Egypt (No.: CU IF 49 22).

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Authors' contributions

All authors contributed equally to this work.

Conflict of interest

The authors declared no conflict of interest.

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