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Histopathological Changes in the Myocardium Caused by Energy Drinks and Alcohol in the Mid-term and Their Effects on Skeletal Muscle Following Ischemia-reperfusion in a Rat Model

ABSTRACT

Background: Although energy drinks have been consumed for many years, their effects on the cardiovascular system continue to be investigated. Today, the most frequently used area of energy drinks is the entertainment sector, and this study investigates the effects of energy drinks and alcohol consumption on rats' limb and myocardium tissue.

Methods: Forty Wistar Albino rats were used and divided into 4 groups. Energy drinks were given to the first group (the energy drink group), alcohol was given to the second group, and energy drinks and alcohol were given to the third group Redbull-Alcohol (RA). Blood samples, leg muscles, and heart tissues were studied after the ischemia–reperfusion model was created at the infrarenal level.

Results: In the histopathological examination of heart muscles, the damage was significantly more severe in the RA group than in the control group ($P < .05$). There was no significant change in the RA group in the limb muscle; however, muscle fiber abnormality was higher. The energy drink group was more prone to carbon dioxide retention and hypoxia, resulting in respiratory acidosis. ($P = .05$). Lactate was significantly higher in the energy drink group ($P = .002$). Glucose concentrations of energy drink and RA groups were higher ($P = .02$).

Conclusion: The high lactate values of the energy drink group and more damaged fibers in the striated muscles in the RA group showed that they are more susceptible to ischemia. Long-term energy drinks and alcohol use may cause damage to the heart muscle and endothelium. Also, the effects of long-term alcohol and energy drink use on the respiratory system should be investigated with more specific studies.

Keywords: Energy drinks, ischemia–reperfusion, alcohol, rat

INTRODUCTION

Energy drinks (EDs) have been produced since 1949, and their use became widespread after the 1980s in western countries.[1,2] Energy drinks entered the Turkish market in the 1990s. Energy drinks are consumed worldwide and in our country without any restrictions. Energy drinks have been consumed widely for almost 40 years, and too much research has been published on human clinical trials and animal models. Although it was introduced to the market as the fluid and energy supplier in sports activities and concentration booster, today's most frequently used area has been the entertainment industry. Because it suppresses alcohol intoxication and hangover symptoms, it allows more alcohol, and its use for this purpose continues to increase and become widespread. High sugar, caffeine, taurine, sodium, and increased alcohol intake may cause cardiac, gastrointestinal, and neurological symptoms.[3] After using these beverages, their service has been questioned worldwide since patients come with these clinical scenarios in a broad spectrum. Based on sporadic case experiences, experiments on animal models have been conducted, and the adverse effects of these beverages on various organ systems have been revealed when used alone or in combination with alcohol.[4-8]

ORIGINAL INVESTIGATION

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Since the early 1980s, many animal experiments have been carried out using these beverages. It has been shown in these experiments that long-term use of EDs reduces the resistance of tissues to ischemia–reperfusion damage, lowers the epilepsy threshold, causes insulin resistance due to the high amount of sugar it contains, and increases alcohol-related side effects as it increases alcohol consumption.[3,4,6,9]

The aim of this study was to show the histopathological changes in the rat myocardium and abdominal aorta by the mid-term use of EDs alone or in combination with alcohol and to compare the histopathological changes at the tissue level with the established ischemia–reperfusion model.

METHODS

Ethics committee approval of the study was received on November 4, 2019, from the same Animal Laboratory Ethics Committee with the decision number of 2019/31. Wistar Albino-type rats were randomly selected with an average weight of 200-300 g bred in this center. The animals were kept in cages in 4 groups under standard room conditions. Forty rats were included in the study, and they were given free access to water and fed with pellet feeds.

Animal Selection

Forty rats were randomly selected and divided into 4 groups for the study. About 1.5 mL/100 g ED (Red Bull) was given to 1 of the groups, 0.486 mg/100 g dose of vodka alcohol to 1 group, and both ED and vodka-type alcohol to another group at the same dose for 1 month. The fourth group was assigned as the control group. The drinks were added to the water consumed by the rats daily. Gavage was not used to avoid stress. We preferred the Red Bull brand as ED because it is the most well-known and consumed brand in the market. We selected vodka-type alcohol as the locally produced Binboa brand is also drunk widely. We preferred vodka because it is the most consumed type of alcohol along with EDs. This protocol was conducted based on previous researches.[10,11]

Pre-experiment Preparation

The feeding of the rats was stopped 12 hours before the experiment. During these 12 hours, water consumption was not restricted. Ketamine at a dose of 60 mg/kg and xylazine at a dose of 5 mg/kg were used as anesthetics. Anesthesia was continued with additional doses if needed. After anesthesia, the animals' thorax, abdomen, and left legs were shaved. Animals were sacrificed after the experiment.

HIGHLIGHTS

- Energy drinks (EDs) and alcohol may impair gas exchange.
- Energy drinks and alcohol may cause hypertriglyceridemia and glycemia.
- Long-term use of EDs and alcohol together showed significant histopathological changes in the myocardium and striated muscle following ischemia–reperfusion.

Surgical Procedure

After anesthesia and shaving, laparotomy was performed, and the peritoneum was opened. The intestines were retracted, and the abdominal aorta was found. An atraumatic bulldog clamp was placed on the abdominal aorta at the infrarenal level. In order to reduce the loss of fluid from the abdomen during ischemia–reperfusion periods, the skin was closed with a single temporary silk suture, and the surgical site was closed with hot wet gas. After 20 minutes of ischemia, the suture was opened, the bulldog clamp was removed, and the skin was approached again during the 20-minute perfusion period. During the waiting period of 40 minutes, the animals were kept at room air and room temperature. After 40 minutes, phlebectomy and cardiotomy were performed after sternotomy under deep anesthesia. There are lots of varieties of ischemia–reperfusion models.[12-16] We chose infrarenal occlusion to reduce the side effects of ischemia on the kidneys and liver. The ischemia–reperfusion period was limited to 40 minutes to avoid respiratory side effects because we kept animals in room air without ventilation. About 1 mL of blood taken by phlebectomy was separated, and blood gas analysis was performed without waiting. The remaining part was transferred to the biochemistry tube and studied in the biochemistry laboratory. After cardiotomy, the abdominal aorta was removed, covering the upper and lower parts of the clamped section. Simultaneously, a sample was taken from the gastrocnemius muscle of the left leg. Tissue samples were stored in formaldehyde solution at +4°C in the refrigerator.

Examination of Blood Samples

First of all, blood samples were studied with Siemens RAPID Point 500 model blood gas device. The pH, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), peripheral oxygen saturation (SpO_2), lactate, hemoglobin, calcium, sodium, and base exchange parameters were studied. The biochemistry parameters of troponin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and cholesterol panel [total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides] were checked. Somogyi–Nelson colorimetric measurement for glucose concentration[17,18] and cholesterol levels were measured by the ferric chloride method.[19] Aspartate aminotransferase and ALT levels were measured by the Reitman and Frankel[20] photocolorimetric method. Troponin levels were measured by the sandwich immunoassay method.

Histological Preparation

Tissues fixed with formaldehyde were kept in cassettes for 1 night at +4°C. They were then washed with tap water for 6 hours. After washing, the tissues were dehydrated with a series of rising alcohol. Tissues were first incubated with 70% alcohol for 1 night and then with 80% (1 hour), 90% (1 hour), 96% (30 minutes + 30 minutes), and 100% (30 minutes + 30 minutes) alcohol. Then they were kept in toluene (15 minutes + 15 minutes) for transparency. Tissues were held in toluene/paraffin (50/50%) mixture for 45 minutes. Then, the tissues taken in pure paraffin were kept in an oven at 60°C for 2 hours and blocked by embedding in hard paraffin.

After trimming the paraffin blocks, they were cut at 4.5 µm thickness with a Leica SM 2010R microtome. The preparations were kept in an oven at 60°C overnight.

Hematoxylin and Eosin Staining

The preparations were deparaffinized with toluene. Then, rehydration was achieved by passing through a series of decreasing alcohol (100%, 100%, 100%, 96%, 90%, 80%, 80%, and 70%) and finally the preparations were washed with distilled water. The preparations were kept in hematoxylin for 15 minutes and were washed in tap water for 15 minutes. Afterward, the differentiated preparations with acid alcohol (1 second) were again kept in tap water for 10 minutes. The preparations, kept in lithium carbonate for 1 minute, were first rinsed in tap water and then in distilled water. Preparations kept in eosin for 1.5 minutes were washed with distilled water. It was passed through an increased series of alcohol for dehydration (70%, 80%, 90%, 96%, 96%, 100%, 100%, and 100%). The preparations were completely removed from the water by soaking in toluene for 30 minutes and were closed with Entellan. Stained slides were evaluated with the Olympus BX61. Histological damages were scored as disorganization, degeneration of the muscle fibers, inflammatory cell infiltration, and vasocongestion (0: normal, 1: mild, 2: moderate, 3: severe) (minimum score: 0, maximum score: 12).[21]

Statistical Analysis

The distribution of variables was classified in the study, and descriptive results were obtained using SPSS version 23 (Statistical Package for the Social Sciences for Windows) program. The normality of data was analyzed using Shapiro–Wilk and Kolmogorov–Smirnov tests. Continuous variables are presented as median with interquartile range (IR). Nonparametric tests were used for significant intergroup results since the number of animals was low. Since the number of groups was 4, the groups were compared using the nonparametric Dunn's multiple comparison test. Another nonparametric Mann–Whitney *U*-test was used for 2-group analysis. A statistically significant difference was accepted with a *P*-value of <.05.

RESULTS

Blood Gas and Biochemistry

Blood gas values studied from intracardiac samples taken after 20 minutes of ischemia and 20 minutes of reperfusion period are shown as median with IR in Table 1. Dunn's multiple comparison test was performed for the values. The lowest pH value was 7.155 mm Hg (IR: 0.14), the highest $PaCO_2$ was 97.45 mm Hg (IR: 37.73), and the most downward PaO_2 was 10 mm Hg (IR: 1.05) in the ED group. *P*-values were .05, .02, and .006, respectively, between all groups. In the control group, results were more close to the normal range for these 3 measurements [the pH was 7.261 (IR: 0.14), $PaCO_2$ was 64.15 mm Hg (IR: 29.97), and PaO_2 was 25.45 mm Hg (IR: 36.38), respectively]. The median SpO_2 was 15% in all experimental groups but 30.4% in the control group (*P*-value: .02).

The highest lactate value was in the ED group: 2.39 (IR: 2.15). The *P*-value was .002 between groups. Hemoglobin levels were higher in the experimental groups than in the control group, but there was no statistically significant difference (*P* = .3). Calcium was not statistically significant between groups (*P* = .45). The highest sodium level was 143.15 mmol/L (IR: 10.55) in the alcohol group, and it was statistically significant with a *P*-value of .009 between groups.

Table 1. Blood Gas Results after Ischemia–Reperfusion

	ED (n = 10)	Alcohol (n = 10)	RA (n = 10)	Control (n = 10)	*P*
pH	7.155 (0.14)	7.245 (0.04)	7.230 (0.07)	7.261 (0.14)	.05
PCO_2 (mm Hg)	97.45 (37.73)	70.3 (7.92)	74.1 (17.73)	64.15 (29.97)	.02
PaO_2 (mm Hg)	10 (1.05)	12.75 (7.48)	15.05 (5.58)	25.45 (36.38)	.006
SpO_2 (%)	15 (0.0)	15 (0.35)	15 (0.05)	30.4 (62.63)	.02
Lactate (mmol/L)	2.39 (2.15)	1.05 (0.88)	1.39 (0.51)	1.58 (0.85)	.002
Hemoglobin (g/dL)	15.5 (1.07)	15.15 (2)	15 (1.65)	14.4 (1.68)	.3
Calcium (mmol/L)	1.13 (0.04)	1.085 (0.15)	1.055 (0.3)	1.19 (0.39)	.45
Sodium (mmol/L)	137.3 (7.7)	143.15 (10.55)	140.55 (3.53)	136.4 (3.6)	.009

Data are shown as median with interquartile range.
PCO_2, the partial pressure of carbon dioxide; PaO_2, the partial pressure of oxygen; SpO_2, peripheral oxygen saturation; ED, energy drink; RA, Redbull-alcohol.

Biochemistry values studied from intracardiac samples taken after 20 minutes of ischemia and 20 minutes of reperfusion period are shown as median with IR in Table 2. The highest glucose value, 381 mg/dL (IR: 59.25), was in the RA group, while the lowest glucose value, 313.5 mg/dL (IR: 124.5), was in the alcohol group. The *P*-value was .02 between all groups. The highest ALT value, 95.25 IU/L (IR: 55.22), was in the control group, while the lowest ALT value, 58.3 IU/L (IR: 32.88), was in the ED group. The *P*-value was .01 between all groups. The highest AST value, 188.8 IU/L (IR: 74.35), was in the control group, while the lowest AST value, 123.75 IU/L (IR: 43.72), was in the RA group. The *P*-value was .05 between all groups.

There was a statistically significant difference between groups in LDL (*P* = .004), but there was no statistically significant difference between groups in triglyceride values (*P* = .08). While the lowest HDL and total cholesterol values were in the RA group, the highest values were in the ED group (*P* = .001 and .004, respectively).

While the highest troponin value was in the control group, the lowest troponin level was in the RA group (*P* = .04).

Histological Examination

No abnormal findings were observed in the control group's microscopic examination of myocardial tissues. Cardiac muscle cells were observed in normal morphology except for inflammatory cells in the connective tissue of the alcohol group. In contrast, cardiac muscle cells with damaged

Table 2. Biochemistry Results After Ischemia–Reperfusion

	ED (n = 10)	Alcohol (n = 10)	RA (n = 10)	Control (n = 10)	*P*
Glucose (mg/dL)	357 (119.5)	313.5 (124.5)	381 (59.25)	320 (104.75)	.02
ALT (IU/L)	58.3 (32.88)	90.1 (77.25)	61.6 (10.65)	95.25 (55.22)	.01
AST (IU/L)	162.15 (85.15)	147.5 (54.88)	123.75 (43.72)	188.8 (74.35)	.05
LDL (mg/dL)	6.4 (4.73)	4.85 (5.73)	3.9 (0.0)	6.85(5.05)	.004
Total cholesterol (mg/dL)	86.5 (27.25)	73.5 (68.75)	46.5 (22.75)	66 (31.25)	.004
Triglyceride (mg/dL)	143.5 (74)	119.5 (105)	172 (173)	101.5 (62.5)	.08
HDL (mg/dL)	82 (37.75)	70 (58.5)	41 (15.75)	60.5 (32.5)	.001
Troponin (ng/mL)	3502.5 (4302.5)	2709 (1710.75)	2469 (1430.75)	4609 (4057.25)	.04

Data are shown as median with interquartile range.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ED, energy drink; RA, Redbull-alcohol.

cytoplasm were observed in some areas in the ED group. Also, eosinophilic heart muscle fibers were observed rarely in the ED group. In the RA group, many inflammatory cell infiltrations and a large number of damages to cardiac muscle cells were observed (Figure 1). Abnormal morphology was observed in the vascular endothelium in some parts of the heart walls.

According to the total histopathological score, the damage is significantly more severe in the RA group than in the control group ($P < .05$, Table 3).

Striated muscle fibers with normal morphology were observed in the control group. Additionally, there were few damaged fibers in alcohol and ED groups. In the RA group, damaged muscle fibers were observed in some areas of the striated muscle tissue (Figure 2). According to the total histopathological score, there is no statistically significant change in the RA group compared to the control group; however, muscle fiber abnormality is higher than in the other groups (Table 4).

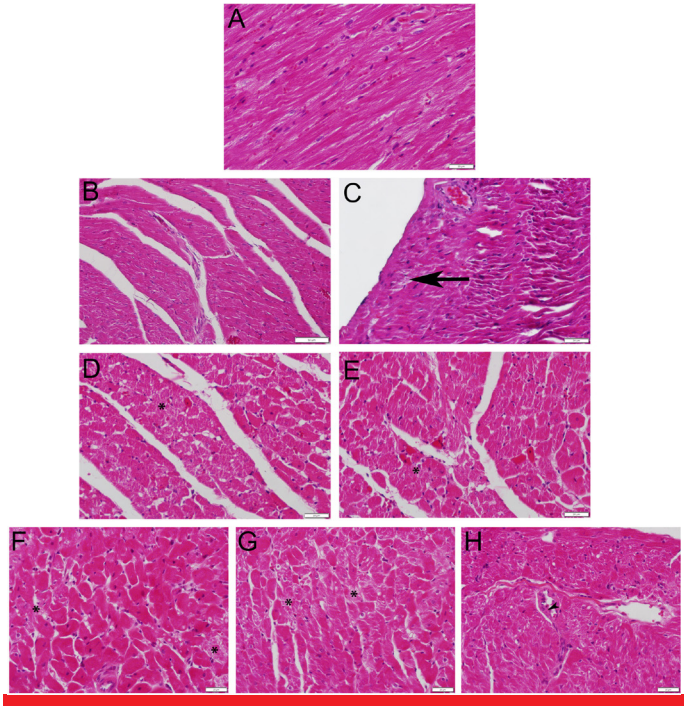

Figure 1. Normal morphology of cardiac muscle in the control group (A); few degenerated fibers (arrow) were shown in Alcohol group (B and C); damaged fibers (*) were seen in ED group (D and E); damaged fibers (*) and abnormal endothelium (arrowhead) were seen in RA group (F, G and H). Bar: 20 µm.

DISCUSSION

This study aims to determine the effects of EDs and alcohol on the rat's heart and muscle tissues following an ischemia–reperfusion model. According to histopathological examination, rats that consumed both ED and alcohol had more damaged cells and abnormal heart and striated muscle tissue structures. Also, all experimental groups were more prone to acidosis, hyperglycemia, and a higher triglyceride level.

To demonstrate the metabolic effects of EDs and alcohol on the organism, we analyzed each animal's blood gas and the abovementioned biochemical parameters. There was no significant difference in the pH value between the control, alcohol, and RA groups. Still, there was a significant difference between ED and the other groups ($P = .05$). The median $PaCO_2$ and PaO_2 were similar between study groups yet worse in the ED group. However, respiratory components were better and statistically significant between the control and study groups. In the literature, there are many human case reports and studies about patients applied to clinics with lung problems such as asthma attacks and bronchospasm after using EDs and beverages containing high sugar and caffeine such as EDs. Varraso et al[22] showed that a high-carbohydrate western diet triggered an asthma attack. Wood et al[23] reported that rapid transition to a high-dose carbohydrate-containing diet adversely affected inflammation in the airways. Since there is no study related exactly to ED and respiratory systems, it may be more accurate to perform such an ischemia–reperfusion model by ensuring airway safety by intubation or tracheostomy so that it does not adversely affect the results of the investigation. In addition, the study of the effects of these beverages on the respiratory system will shed light on future studies.

Another most striking value among the blood gas parameters was lactate. The median value for lactate in the ED group was 2.39 mmol/L (IR: 2.15), and the *P*-value was found to be

Table 3. Histopathologic Score of Cardiac Muscle in Control, Alcohol, ED, and RA Groups

Groups	Disorganization	Degeneration	Inflammation	Vasocongestion	Total Score
Control	0	0	0.5	0.5	1
Alcohol	0.5	0	2	0	2.5
ED	1	1	2	0	4
RA	1	2	3	2	8*

ED, energy drink; RA, Redbull-alcohol.

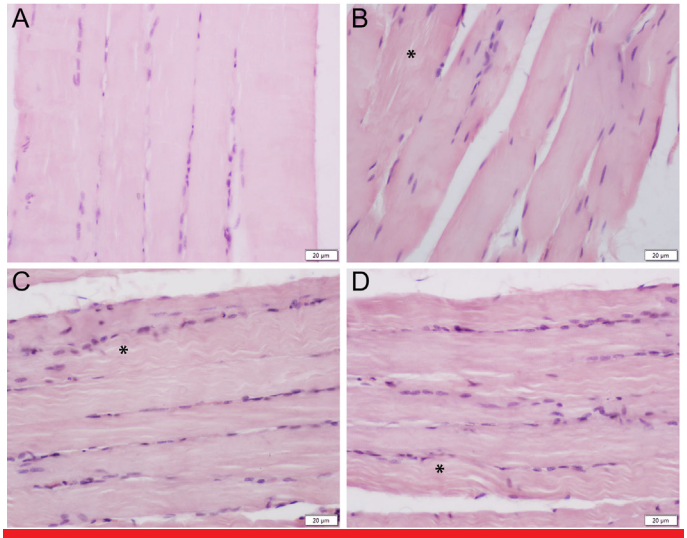

Figure 2. Normal morphology of striated muscle (A); few degenerated fibers (*) were shown in the alcohol group (B), the ED group (C), and the RA group (D). Bar: 20 µm.

.002 between groups. In the double-blind study conducted by Ferreira et al[24] on 14 healthy adult males, the subjects were divided into 4 groups, just as in our study. The subjects underwent an exercise protocol, and at this stage, their oxygen consumption, ventilation, and respiratory parameters were recorded by ergospirometry, and their blood lactate levels were measured. Lactate levels (30 minutes after drug ingestion, 30 and 60 minutes after the effort test) were higher in the alcohol and alcohol + ED sessions compared with the control session. A double-blind experiment conducted by Lara et al[25] with 14 young adult male swimmers separated the groups into placebo and EDs. They found that the blood lactate levels were statistically significantly higher in the ED group after the exercise protocol.[25] The increased lactate value resulting from ischemia-induced anaerobic glycolysis suggests that the ED group is more affected by ischemia.

Energy drinks contain high sodium and calcium, which are essential at the cellular level in ischemia–reperfusion injury; therefore, we studied these 2 electrolytes. Calcium was more elevated in the control group than in the other groups, but it was not statistically significant. We could not find any direct or indirect study on calcium levels related to EDs in the literature. Since calcium is an essential element in myocardial and muscle contractility and hemostasis as factor 2, we found it worth writing the results in our study. As expected, the sodium value in the control group was lower than all the other groups, and the highest median values were in the alcohol and the RA group. For many years, it has been known that diets with high sodium content cause intravascular volume overload and hypertension by increasing the plasma sodium level.[26]

Glucose level was higher in the ED and RA group than in the other groups. Although median glucose values were similar between the alcohol and the control group, they were slightly lower in the alcohol group. Since alcohol consumption inhibits both gluconeogenesis and glycolysis, it creates hypoglycemia when used alone, while it can create hyperglycemia when consumed with foods containing high carbohydrates.[27] We think that the increased glucose level in all groups, including the control group, is due to increased sympathetic activity triggered by acidosis and surgical procedures.

Many studies have shown that AST and ALT activities increased at the serum level and decreased at the tissue level in animal groups that received alcohol, ED, and both. Mihailović et al[28] showed that AST and ALT activities increased at the serum level and decreased at the tissue level in their experiment with 8 animals, each separated into alcohol and control groups. Munteanu et al[11] reported similar results with 28 Wistar Albino male rats. They divided the animals into 4 groups, as in our study, and after 30 days of treatment, they attached 10% of their weight to their tails and put them into a challenging swimming protocol. They showed that AST and ALT activities decreased at the tissue level and increased at the serum level at the end of the exercise. Due to the ischemia–reperfusion model we established in our study, our results may not be similar to these studies.

Table 4. Histopathologic Score of Limb Muscle in Control, Alcohol, ED, and RA Groups

Groups	Disorganization	Degeneration	Inflammation	Vasocongestion	Total Score
Control	0	0	0	0	0
Alcohol	1	0.5	0	0	1.5
ED	1	1	0	0	2
RA	1	2	0.5	0	3.5

ED, energy drink; RA, Redbull-alcohol.

Energy drinks cause changes in cholesterol levels in long-term use due to the vitamin niacin and caffeine. Many human and animal studies have been published on these substances. Voskoboinik et al[29] published a review on the effects of caffeine on the cardiovascular system in 2018. This study emphasized that short-term consumption of unfiltered coffee increased serum triglyceride, cholesterol, and LDL levels. A meta-analysis of 12 randomized trials, including 1017 people who consumed coffee for an average of 45 days, reported that total cholesterol, triglyceride, and LDL levels increased significantly. Still, HDL levels did not change significantly.[30] These effects were primarily seen in people who consumed more than 6 cups of unfiltered coffee daily and had a poor lipid profile.

The effect of alcohol use on the lipid profile varies greatly with the amount and duration of consumption. Wang et al[31] reported a study that included 8 animals in the alcohol and control group; they showed increased cholesterol, HDL, and LDL levels in medium-term alcohol use. Our study found that the alcohol group had increased total cholesterol, HDL, and triglyceride levels but decreased LDL levels compared to the control group.

Another substance that affects cholesterol and triglyceride levels is the vitamin niacin added to ED. Niacin can be used alone or in addition to statin therapy to lower the total cholesterol, LDL, and triglyceride levels and increase HDL levels in individuals with impaired lipid profiles and have risk factors for cardiovascular events. Although the Impact on Global Health Outcomes (AIM-HIGH) and The Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) studies have shown that niacin does not reduce cardiovascular events, it is one of the prominent substances in ED marketing today. Munteanu et al[11] administered ED and ethanol to rats separately and together and compared these 3 groups with the control group. They found that total cholesterol levels in all groups were statistically significantly lower than in the control group.[11] Our study found that the ED group had lower LDL levels and higher cholesterol and HDL levels than the control group. We also saw that triglyceride levels increased approximately 1.5 times compared to the control group. We found that triglyceride levels were about 1.7 times higher, and total cholesterol, HDL, and LDL levels were lower in the RA group than in the control group.

Munteanu et al[11] published a study on ED and alcohol. They found signs of alcoholic cardiomyopathy (poor arrangement of myofibrils and swollen mitochondria with dilated crystals) in the hearts of alcohol-treated rats. They also observed dilated crystal swollen large mitochondria and abnormal spaces between myofibrils in the ED group, which were thought to be caused by oxidative damage.[11] Mansy et al[32] compared 3 groups of animals given ED at 3 different doses with the control group. They found that serum antioxidant enzyme levels were significantly lower in animals with medium and high ED amounts. Their histopathological examination of the liver and kidney observed congestion and necrosis in the cells and inflammation in the intercellular tissues.[32] Reis et al[4] divided the animals into 6 groups: control, low-dose ED, high-dose ED, ethanol, ethanol and low-dose ED, and ethanol and high-dose ED groups in their animal experiment study. Liver tissue samples of the rats were examined, and the findings of balloon degeneration and lobular inflammation were categorized and evaluated at the end of the 15-day study. As a result, these pathological findings were mostly detected in rats given high-dose ED and high-dose ED with ethanol. In our study, multiple inflammatory cell infiltration and multiple damages were observed in the cardiac muscle cells of animals in the RA group. Abnormal structuring was observed in the vascular endothelium in some parts of the heart walls. In the striated muscle cells, more damaged fibers were observed in the RA group compared to the other groups.

Study Limitations

Although respiratory acidosis was statistically significant in the ED group than in the control group, performing an experiment using tracheal ventilation would be more accurate. Although we were ensured that all rats had finished their daily drinks, measurement of blood ethanol level could be more appropriate. Also, using electron microscopy would be more useful in histopathological examination.

CONCLUSION

In conclusion, the consumption of ED and alcohol may cause respiratory acidosis in rats. The high lactate values of animals using ED suggest that they are more affected by ischemia. Observing more damaged fibers in the striated muscles in the ED and alcohol group also supports this situation. More damaged cells in the heart muscle, inflammatory cell infiltration in the connective tissue, and abnormal structuring in the vascular endothelium indicate that long-term ED and alcohol use may cause damage to the heart muscle and endothelium.

Ethics Committee Approval: İstanbul Experimental Research Development and Education Center (İDEA) Ethics Committee approved this study on 04/11/2019 with the number 2019/31.

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