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Effects of repeated low and high doses of energy drink on kindling-type seizures, antidepressant and anxiolytic treatment in mice

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ABSTRACT

Energy drinks (EDs) may cause neuropsychological risks. This study scrutinized the effects of repeated low and high-dose energy drinks (LED; 4ml/kg and HED; 12ml/kg) exposures on total locomotor activity (TLA), seizure activity, and antidepressant and anxiolytic treatments. 144 female Balb-C mice were randomly divided into three groups (n=48) named seizure, depression, and anxiety. Then, animals were divided into six subgroups. TLA was measured in all groups. While the epileptic activity was tested in intraperitoneally (i.p.) administrated pentylenetetrazole (PTZ)-induced kindling mouse model, depression and anxiety experiments were tested with citalopram (CT) and alprazolam (AL)-pretreated mice. HED decreased the TLA more than the LED group. Seizures in the LED group were higher than in the water drink (WD) group. HED changed depressive outcomes in the HED group. LED and HED altered anxiogenic outcomes in both groups. Conclusively, LED and HED increased the seizure frequency, decreased the success of depression and anxiety treatments.

Keywords: anxiety, behavioral changes, depression, energy drink, seizures

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INTRODUCTION

Energy drinks (EDs) are consumed to improve or increase performance levels during fatigue or exercise, to keep awake in needed situations, to increase endurance, to treat insomnia or cognitive performance during exams, or to enhance the performance of alcohol by mixing it with alcohol. Statista Research Department reported, "In 2023, the annual consumption volume of EDs averaged approximately 29.19 liters in the United States. The United Kingdom is in second place with 13.2 liters, while Russia is in last place with 0.29 liters, a difference of 28.9 liters from the ranking leader, the United States[1]". While so much consumption of the ED has been reported that they interact with drugs or treatments and can cause psychological and other problems, such as cardiovascular, etc., during treatment in the literature[2-4].

The major constituents in most energy drinks are caffeine (1,3,7-trimethyl-xanthine), taurine (2-aminoethanesulphonic acid), and glucose. However, they may contain various ingredients such as ginkgolides, ginseng, L-carnitine, guarana, milk thistle, some vitamins[5,6].

Following the intake of caffeine, the major active component of EDs, it is absorbed from the gastrointestinal tract approximately 90-99%[7]. The pharmacological effects of caffeine occur by modulation of the neurotransmission of dopamine. Furthermore, stimulant effects related to neuronal pathways result in the central and peripheral nervous system, including modulation of behaviors such as attention, alertness, arousal activity, and locomotor behaviors[8].

Epilepsy is a common neurological illness. It is characterized by abnormal electrical activity, resulting in seizures and/or unusual behaviors, feelings, and sometimes loss of major consciousness. It has negative neuropsychological, cognitive, and social outcomes[9]. The previous studies stated that the regular intake of ED increased epileptic seizures via the toxic impacts of taurine and caffeine[10,11].

Major depressive disorder (MDD) and generalized anxiety disorder (GAD) are the primary causes of disability and premature mortality. At the global level, it is estimated that over 300 million individuals are affected by both MDD and GAD[12]. In recent years, many studies reported that EDs may contribute to anxiety[13-17]. All of these have been displayed to be a positive association between ED consumption and the development of neuropsychiatric disorder symptoms.

The present study aimed to investigate the effects of repeated low and high doses of ED consumption on epileptic seizures, total locomotor activity, and the success of antidepressant and anxiolytic treatments in mice.

METHODOLOGY

Animals

One hundred forty-four (n=144) female Balb-C (7 ± 2 weeks old, 20 - 30 g) mice were provided from the Experimental Animal Centre of Yeditepe University. Animals were housed in a 12:12-hour reverse dark-light cycle to allow water exposure and energy drink intake studies to be conducted during each animal's active light cycle. Animals were maintained at a constant temperature of 22 ± 2°C and fed with standard chow diet and water *ad libitum* throughout all experiments. First, one hundred forty-four animals were randomly separated into three groups (forty-eight (n=48) animals in each group) for the main experimental studies: a) depression, b) anxiety, and c) seizure. Then, animals in each main experimental group were divided into six (n=6) subgroups. All subgroups included eight (n=8) animals.

The study had approval from the local animal ethics committee of Yeditepe University (16.02.2024/427). All experiments were done in line with relevant principles and institutional guidelines of the Local Animal Ethical Committee and performed in the Faculty of Pharmacy, Department of Pharmacology Research Laboratory. This study was reported under the ARRIVE guidelines.

Chemicals

The most commonly consumed energy drink was chosen and purchased from a local market (Istanbul, Turkey). It was administered through oral gavage. The main stimulant components are taurine (4.2 g/L), glucuronolactone (2.55 g/L), and caffeine (350 mg/L). Alprazolam (AL), citalopram (CT), and pentylenetetrazol (PTZ) were purchased from a chemical company (Sigma Aldrich).

The protocols of experimental tasks

Energy drinks (ED) and water drink (WD) were administered (low dose=4 ml/kg; LED and high dose=12 ml/kg; HED) twice per day (at 08:30 h and 15:30 h) by oral gavage and mice were exposed to chronic LED, HED and WD for 15 days, and all tests were performed between 09:00 am and 16:00 daily. While HED included 0.05 g taurine, 0.03 g glucuronolactone, and 0.0042 g caffeine, LED included 0.016 g taurine, 0.01 g glucuronolactone, and 0.0014 g caffeine. Before energy drink exposure, mice were administered intraperitoneally (i.p.) citalopram (15 mg/kg) and alprazolam (0.5 mg/kg) for antidepressant and anxiolytic treatments.

Total Locomotor Activity (TLA) of animals was measured with a locomotor activity monitoring system (MAY 9908 & 0107 model – Activity Monitoring System – Commat Ltd., TR) according to the test developed before[18]. After

the administration of the ED to the pretreated mice, the mice were placed into an activity chamber for three days and 10 minutes, following 5 5-minute habituation period. Experiments were performed in a soundproof laboratory room, and TLA of all mice was measured in the first three days and on day 15 after energy drink administration.

Sequential intraperitoneal (i.p.) injections of pentylenetetrazol (PTZ) subconvulsive doses caused the development of a kindling epilepsy model. However, repetitive low-dose injections of PTZ reduce the threshold to evoke a convulsive seizure[19]. After performing the TLA, PTZ was given (i.p.) every other day in three different (17, 35, and 70 mg/kg) doses, respectively. Following the injection of PTZ, mice were observed and evaluated for 30 minutes for the occurrence of clonic or other seizure types. The seizure severity and duration were evaluated and scored according to the Racine scale[20].

Porsolt's Forced Swim Test (FST) was conducted according to the method developed before[21]. 15 mg/kg citalopram-pretreated mice were placed into the water and forced to swim for 10 minutes. The immobility times were monitored and recorded by a computer system. Following the test, mice were removed from the water, carefully dried with paper towels, and then they were returned to their home cages.

Elevated-Plus Maze (EPM) test was done according to the method developed by Pellow and colleagues[22]. The explorative behaviors of mice were tested on day 15, in all ED and WD-administered and already 0.5 mg/kg alprazolam-pretreated mice. The EPM consists of two open and two closed elevated plexiglas arms that form a plus. When mice are anxious, mice prefer to enter closed, dark arms. At the beginning, each mouse was placed in the center of the plus maze facing an open arm. The number of entries of mice into each type of arm and the time spent in each arm were recorded for 5 minutes and analyzed with the EthoVision XT system (Commat Ltd., TR). At the end of the experiments, the animals were primarily kept in rehabilitation and then sacrificed.

Statistical analysis

Data were analyzed by the SPSS Statistics program v.25 (Chicago, IL, United States). Data were presented as mean ± standard error of mean (SEM). $p<0.05$ was accepted as significant. To compare two groups, Student's t-test was used. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to analyze differences between groups.

RESULTS and DISCUSSION

The effects of ED on total locomotor activity

Total locomotor activities (TLA) among the WD, LED, and HED groups showed significant differences ($F(2,69)=15.906$). Findings indicated significant differences between WD andHED ($p \leq 0.001$) and L ED and HED ($p \leq 0.05$) groups (Figure 1).

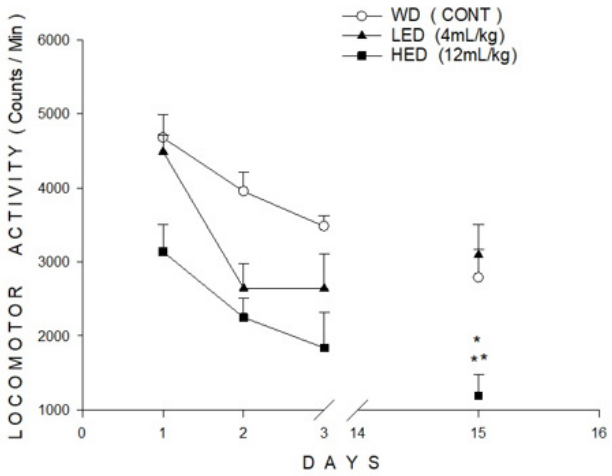

Figure 1. Time course changes in Total Locomotor Activity of ED exposure in mice for 10 minutes. Eight (n=8) animals in each group. Error bars represent ± *SEM*; LED: Low Energy Drink; HED: High Energy Drink; WD: Water Drink. To compare two groups, Student's t-test was performed. To compare all groups with each other, ANOVA was followed by Tukey's Post-hoc test. *$p<0.05$; different from the LED group; *$p<0.001$, different from the WD group.

There was also a significant difference in TLA between time points across the four days (on days 1, 2, 3, and 15) ($F(3, 68)=18.1$, $p \leq 0.01$). The TLA of the HED group was significantly lower than the LED and WD groups at the beginning. The decreased TLA of HED groups continued until the end of the consumption period.

The provocative effects of repeated ED consumption on seizure

The repeated administration of subconvulsive PTZ (17, 35, and 70 mg/kg) on every second day (for 15 days, 7 injections) resulted in a gradual increase in seizure score in PTZ-pretreated WD or LED groups (Figure 2). The HED was not used with PTZ pre-treatment to reduce animal distress (mortality) and stayed within the limits of animal research ethics guidelines. 17 mg/kg PTZ-pretreated LED group revealed a significant difference between the seizure scores of LED and WD groups [exposure $F(1, 70)=42.97$, $p<0.01$] (Figure 2A).

In addition, the 17 mg/kg PTZ-pretreated LED group showed a difference in the seizure scores among the day intervals [day $F(7, 70)=23.67$, $p<0.01$]. 35 and 70 mg/kg PTZ-pretreated LED groups exhibited significant differences between the seizure scores of LED and WD groups [exposure $F(1, 70)=24.89$, $p<0.01$] (Figure 2B) and [exposure $F(1, 36)=15.64$, $p<0.01$] (Figure 2C), respectively. Moreover, 35 mg/kg PTZ-pretreated LED group essentially increased the seizure scores on days 7, 9, 11, 13, and 15 relative to the WD group ($t=4.99$, $p<0.01$). The same group also demonstrated a significance in the seizure scores among the day intervals [day $F(7, 70)=31.367$, $p<0.01$]. Furthermore, in the 70 mg/kg PTZ-pretreated LED group, significantly high seizure scores were observed on days 3, 5, 7, and 9 when compared to the D group ($t=3.5$, $p<0.01$). However, seizure scores could not be shown after day 9 due to the death of all animals in the WD group pretreated with PTZ 70 mg/kg (Figure 2C).

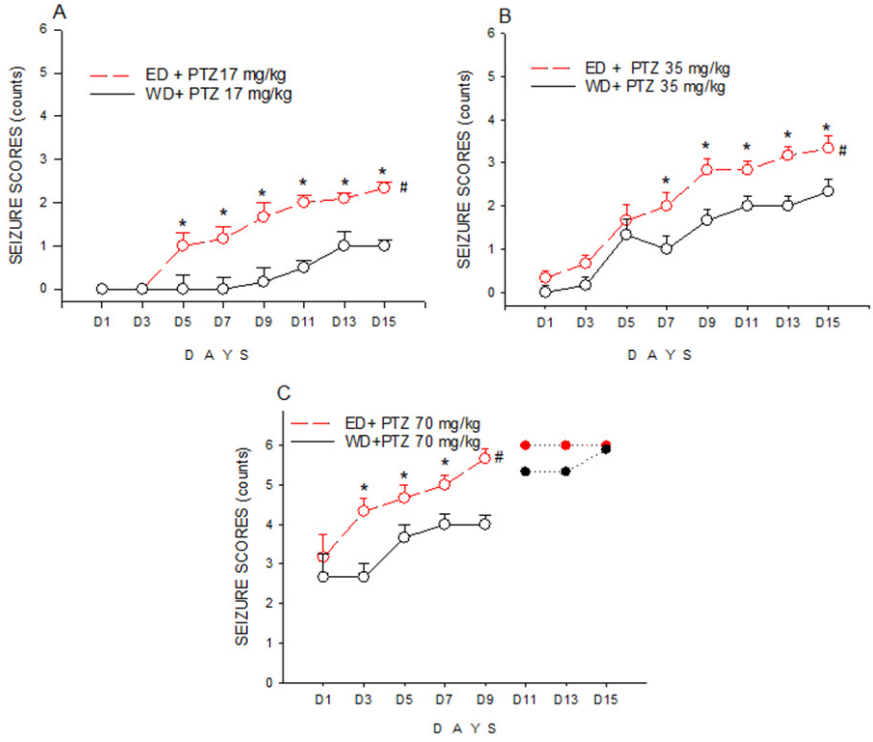

Figure 2. The effects of low dose ED or WD exposure on the seizure scores in mice with administration of (A) 17 mg/kg, (B) 35 mg/kg and (C) 70 mg/kg doses of PTZ on every second day for 15 days. Eight (n=8) animals in each group. Error bars represent ± *SEM*; ED: Energy Drink; PTZ: Pentylenetetrazol; WD: Water Drink. To compare two groups, Student's t-test was performed. To compare all groups with each other, ANOVA was followed by Tukey's Post-hoc test. *$p<0.05$, different from the WD group; #$p< 0.01$, different from the LED group.

As shown in Figure 3, the effect of LED on seizure durations increased on days 1, 7, and 15 in the groups pretreated with PTZ (17 and 35 mg/kg). Significant difference was determined between the seizure durations of LED and WD groups [$F(1, 28)=26.19$, $p<0.01$]. The seizure durations increased significantly in the PTZ 17 mg/kg pre-treated LED group on days 7 ($p=0.02$) and 15 ($p<0.05$) when compared to the WD (Figure 3A). Moreover, findings demonstrated significant increased seizure durations in LED+PTZ (35 mg/kg) group on days 7 and (p<0.01) and 15 ($p<0.01$) when compared to the WD+PTZ (35 mg/kg) group (Figure 3B).

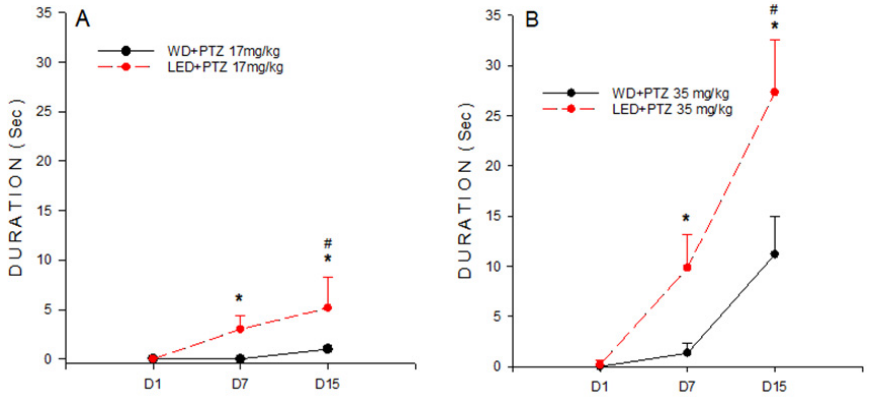

Figure 3. The effects of LED or WD exposures on the durations of PTZ-induced kindling seizures with doses of (A) 17 mg/kg. (B) 35 mg/kg in mice on days 1, 7, and 15. Eight (n=8) animals in each group. Error bars represent ± *SEM*; LED: Low Energy Drink; WD: Water Drink; PTZ: Pentylenetetrazol. To compare two groups, Student's t-test was performed. To compare all groups with each other, ANOVA was followed by Tukey's Post-hoc test. *p<0.05, different from the WD group; #p<0.05, different from the LED group on day 7.

The effects of ed exposure on treatment of depression

The effects of ED on TLA and immobility performances were tested in WD, LED, and HED groups on day 15, respectively (Figure 4A, B). CT pre-treatment did not change the TLA levels of the WD and LED groups on day 15, but caused a significant reduction in the TLA levels of the HED group (treatment [$F(3, 16)=8.03$, $p<0.01$]) (Figure 4C). The immobility times were determined significantly increased only in the HED group on day 15 when compared to the WD and LED groups [exposure [$F(2, 12)=34.803$, $p<0.01$], but mice in the LED group exhibited a significant reduction in immobility time when compared to the WD group. The increased immobility time of the HED group was significantly higher than the WD and LED groups (Figure 4B). Furthermore, a significant reduction was exhibited [$F(3, 16)=6.441$, $p<0.01$] in the immobility time of the CT-pretreated WD group, but not in those of the LED and HED groups (Figure 4D).

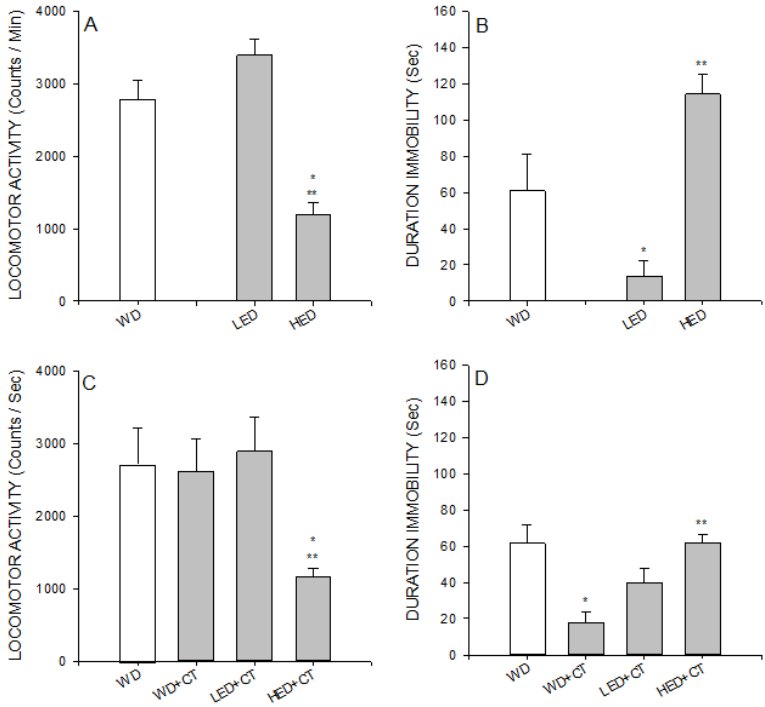

Figure 4. The effects of ED exposure and CT on TLA and immobility time performances in two different doses of ED or WD exposed mice. (A) The effects of ED exposure on TLA. (B) The effects of ED exposure on immobility time performances. (C) The effects of CT on TLA. (D) The effects of CT on immobility time performances. Eight (n=8) animals in each group. Error bars represent ± *SEM*; CT: Citalopram, LED: Low Energy Drink; HED: High Energy Drink; WD: Water Drink. To compare two groups, Student's t-test was performed. To compare all groups with each other, ANOVA was followed by Tukey's Post-hoc test. *p<0.05, different from the WD group; **p< 0.01, different from the LED and the LED+CT groups.

The effects of ED exposure on anxiolytic treatment

TLA and EPM performances were tested on day 15 of ED exposure after the treatment periods of AL (Figure 5). HED significantly reduced the TLA on day 15 in mice than those of the WD nd LED group [$F(2, 12)=7.180$, $p<0.01$] (Figure 5A).In addition, the time spent in open arms was reduced in the LED group (Figure 5B), and the time spent in closed arms essentially increased in the LED and HED groups (Figure 5C). As shown in Figure 5D, TLA levels of the HED+AL group were significantly lower than other groups on day 15 [$F(3, 16)=13.003$, $p<0.01$]. Significant difference among the time spent by the groups in the open arm [$F(2, 12)=5.15$, $p<0.01$] and closed arm [$F(2, 12)=4.369$, $p<0.05$]. There were significances among the treatment groups: time spent in the open arm [$F(3, 16)=5.175$, $p<0.01$] (Figure 5E) and in the closed arm [$F(3, 16)=16.6369$, $p<0.01$] (Figure 5F). Moreover, AL pre-treatment significantly decreased the time spent in the closed arm in all treatment groups ($p<0.05$).

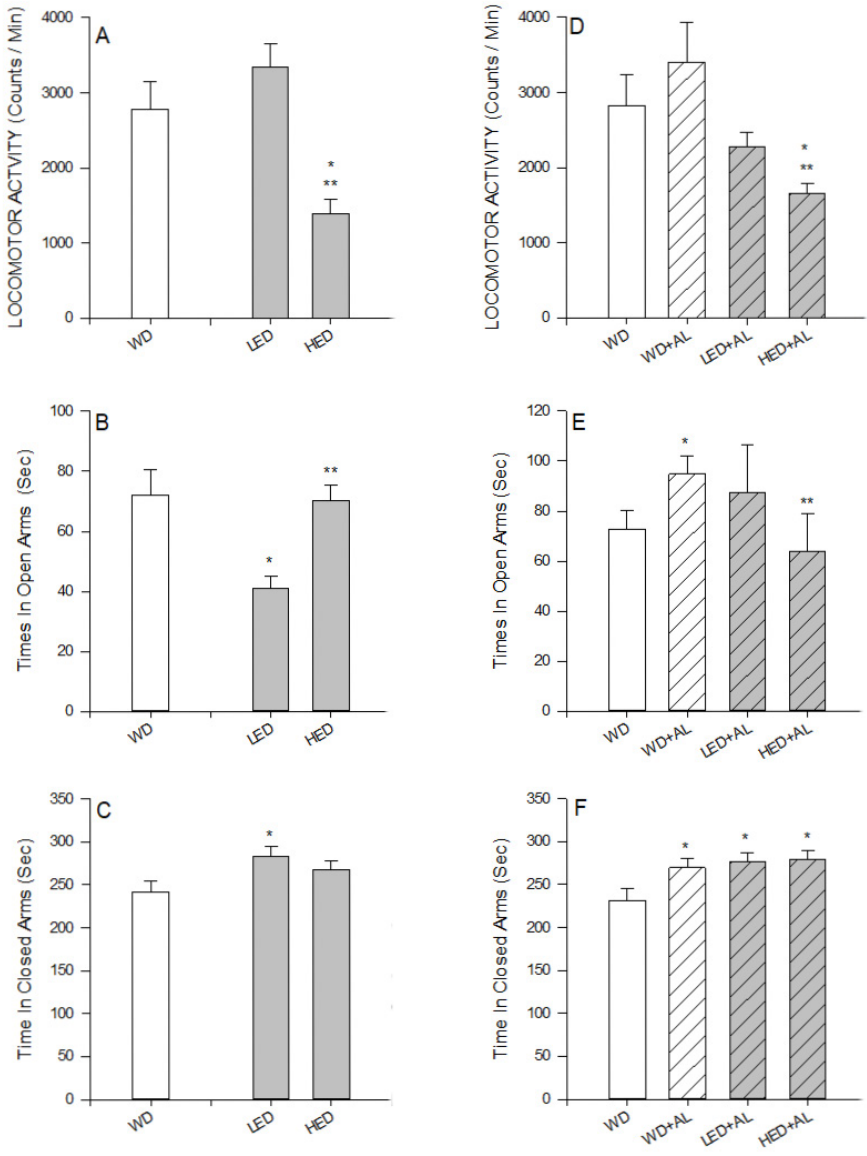

Figure 5. The effects of ED exposure and AL on TLA and on the anxiogenic behaviors (by the EPM test) in two different doses of ED or WD exposed mice on day 15. (A) The effects of ED exposure on TLA. (B) The effects of ED exposure on time spent of mice in open arms. (C) The effects of ED exposure on time spent of mice in closed arms. (D) The effects of AL on TLA. (E) The effects of AL on time spent of mice in open arms. (F) The effects of AL on time spent of mice in closed arms. Eight (n=8) animals in each group. Error bars represent ± SEM; AL: Alprazolam; LED: Low Dose Energy Drink; HED: High Dose Energy Drink; WD: Water Drink; EPM: Elevated Plus Maze. To compare two groups, Student's t-test was performed. To compare all groups with each other, ANOVA was followed by Tukey's Post-hoc test. *p<0.05, different from WD; **p< 0.01, different from LED and LED+AL groups.

The current study showed that repeated ED consumption worsened total locomotor activity, increased the severity and duration of seizures, and decreased the success of antidepressant and anxiolytic treatments. It has been well known that the main ingredient found in energy drinks is caffeine[23], which is considered as associated with locomotor performances, explorative, anxiogenic, and antiepileptic behaviors[24]. EDs as trigger factors for seizures had been presented in pediatric patients in a case report[25]. Our study was designed to mimic the possible clinical conditions of the patients to trigger and reveal the potential seizure occurrence. At the beginning of ED exposure, we observed that LED (4 mL/kg) alone did not trigger the progression of kindling as evidenced by the increase in seizure score as compared to the WD. However, the repeated administration of subconvulsive PTZ doses (17, 35, and 70 mg/kg) produced gradual increases in seizure scores in WD and LED groups. LED facilitated triggering seizure when compared to the WD group, and a significant difference was observed with PTZ (17 mg/kg) in the LED group on the fifth day.

As is known, several neurotransmitters and neuromodulators are released during seizures in animals and humans, including adenosine, which greatly increases as a neuromodulator and an endogenous anticonvulsant in association with seizures[26]. Similar to the previous study, our findings suggest that caffeine, an adenosine receptor antagonist, might not antagonize the susceptibility to seizures at the beginning of ED exposure. However, it antagonized in long-term exposure and in high doses[27]. ED and/or their contents exposure may be associated with seizure and increased the severity and duration of seizure, as shown in our study and clinical case reports[15]. Energy drink used in this study included more taurine (4.2 g/L) than caffeine (350 mg/L). Thus, the amount of ingredients also affected the results of seizure studies. By repeated ED exposure, disturbances in the modulation of adenosine action could be implicated in the increase of the susceptibility to seizures.

It had been known until 1981 that caffeine induced seizures in 50% of mice[28]. In a previous study, single low (10 mg/kg) and high dose (20 mg/kg) i.p. caffeine was given to the rodents, and findings exhibited that high dose caffeine led to an increase in duration and frequency of seizure[29]. In another study, investigating the effect of energy drinks alone and in combination with alcohol on epileptic activities, it was reported that ED and alcohol significantly increased the severity of epileptic seizures[30]. These findings suggested a parallelism with the increase in epilepsy duration in our study. In contrast to our study, two weeks of caffeine exposure with 6 mg/kg (p.o.) and 60 mg/kg PTZ administration (i.p.) were reported to show that long-term caffeine consumption decreased

the duration of PTZ-induced seizures in adult male rats[31]. The reason for these unexpected findings may be related to differences in ED consumption frequency and the amount of active ingredients in various EDs.

A previous study showed that high caffeine doses altered cognitive functions and social behavior of the mice by changing anxiety and sociability behaviors[32]. It was estimated to be associated with the biphasic effect of caffeine on locomotion of mice by stimulating locomotion at 6.25–25 mg/kg–1 i.p. doses, while depressing it at 100 mg[33,34]. Our study also had a biphasic behavioral response in immobility time related to the dose of repeated ED consumption. On the other hand, the findings of the forced swimming test exhibited that CT pretreatment (15 mg/kg) significantly decreased immobility time in only the HED group when compared to the WD.

Similarly, it was suggested that the forced swimming test and tail suspension test resulted in a reduction in immobility time in mice treated simultaneously with antidepressants[35]. It may be related to the antidepressant effect of citalopram and/or the dose of caffeine found in LED. When caffeine is consumed moderately, it has an excellent safety profile. However, long-term ingestion can lead to undesirable CNS effects as well as pharmacological tolerance[27]. In this study, LED did not essentially change TLA, while HED caused an increase in TLA. This makes it hazardous since it can be consumed by everyone for long-term consumption. It can be suggested that the mice administered HED had the lowest TLA compared to the WD and LED groups. Our results are also consistent with the findings of other studies, which showed that moderate or high caffeine levels reduced TLA in adult rodents[36,37]. It has also been demonstrated that the regular or low-dose caffeine antagonized the A2a receptors and resulted in psycho-stimulation[33] while a high caffeine dose resulted in A1 antagonism, which caused psychomotor depression, or it neutralized the psycho-stimulation induced by low-dose caffeine action on A2a receptors[34]. As shown in the depression findings of the current study, CT pretreatment showed a beneficial effect and prevented changes in TLA scores in LED and HED groups, when compared to the WD and other groups. As expected, CT pretreatment decreased depression-like behaviors or immobility time in WD groups.

Furthermore, a study exhibited that long-term caffeine administration may have an anxiolytic effect as well as an antidepressant effect, and the reason for this was reported as the increase of dopamine and serotonin levels in the hippocampus[38]. In our study, LED showed an increased TLA while HED was vice versa. However, the anxiogenic effects were observed in LED but not in

HED-exposed mice, interestingly. The findings of previously performed two studies indicated that acute and chronic administration of caffeine produced an anxiogenic-like effect by the highest and low doses (30 mg/kg, i.p. and 5 mg/kg orally) in mice, respectively[33,39].

Similar to the findings in Kaplan et al., decreased anxiogenic effects by HED in mice could not explicate by the anxiogenic effects of caffeine and taurine in our study. The other behavioral effects of caffeine and taurine, such as the antidepressant effect, can mask the anxiogenic effects of HED in mice[39].

AL pretreatment (0.5 mg/kg, i.p.) prevented the increment of anxiogenic effects caused by LED consumption in the LED+AL group. Our study displayed that ED (caffeine plus taurine) changed anxiety treatment outcomes during the chronic ED consumption period in mice. Caffeine consumption greater than 250 mg/day can produce physical symptoms including restlessness, irritability, anxiety, nervousness, and insomnia[40]. It is clinically important to consider ED consumption when evaluating treatments related to mood or anxiety disorders[13]. Our results revealed that clinicians needed to be educated to inform their patients about ED effects, particularly during treatment.

Limitations

The study has some limitations. First of all, the study was carried out in a short time. It could have been done for a longer period of time, such as one or two months, in order to obtain more valuable findings in terms of antidepressant and anxiolytic treatment studies. It would also be better to add an intermediate dose as well as low and high doses of ED. Since the ethics committee did not allow the use of more animals, an intermediate dose could not be added. It is planned to use intermediate doses in future studies.

In conclusion, concomitant consumption of EDs provoked the kindling-type epileptogenesis in terms of frequency and duration in mice. In addition, the success of antidepressant and anxiolytic treatments, respectively with citalopram and alprazolam, decreased with the repeated consumption of EDs during treatments, and altered the behavioral outcomes. Total locomotor activities of all experimental animals were impaired and affected negatively via repeated ED consumption. The obtained findings may be significant and instructive in terms of shedding light on future clinical studies. These findings can be further investigated in various clinical settings.

STATEMENT OF ETHICS

The study had approval from the local animal ethics committee of Yeditepe University (16.02.2024/427).

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Supervision, TC; conceptualization, TC, CM; design, CM, TC, KY, SBKY, GD; chemical and resources, CM, TC, KY, SBKY, GD; data collection and processing, CM, TC, KY, SBKY, GD; literature search, CM, TC, KY, SBKY, GD; analysis and interpretation, CM, TC; writing, CM, TC, GD. All authors read and approved the manuscript.

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