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Effects of Long- and Short-term Consumption of Energy Drinks on Anxiety-like, Depression-like, and Cognitive Behavior in Adolescent Rats

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Purpose: The purpose of this study was to understand the impact of long- and short-term energy drinks on anxiety-like, depression-like, and cognitive behavior in adolescent rats. **Methods:** Adolescent rats (age six weeks) were randomly classified into a control group (CON), a long-term administration group (LT), and a short-term administration group (ST). The LT group was orally administered 1.5 mL/100 g (body weight) of energy drink twice daily for 14 days, the ST group was orally administered for one day, and the control group applied the same amount of normal saline. Later, an open-field test, a forced swim test, novel object recognition test, and an 8-arm radial maze test was conducted to assess the rats' anxiety, depression, and cognitive function. **Results:** There were different effects in the long- and short-term groups of energy drink administration. In the LT group, anxiety- and depressive-like behavior increased because of increased movement in the side corner and decrease of immobility time. Also, the time to explore novel objects decreased, and the number of correct responses was reduced, indicating a learning and memory function disorder. However, the ST group was not different from the control group. **Conclusion:** These results indicate that long-term consumption of energy drinks can increase anxiety-like, depression-like behavior, and this can lead to decrease in learning and memory functions. Thus, nurse and health care providers should understand the impact of energy drink consumption in adolescence to provide appropriate practices and education.

Key Words: *Energy drinks; Adolescent; Anxiety; Depression; Cognitive dysfunction*
국문주요어: 에너지음료, 청소년기, 불안, 기억, 인지

INTRODUCTION

Energy drinks (EDs) are a type of functional beverage advertised to help improve concentration and recover from fatigue. Since the release of Red Bull in the U.S. in 1997, it has continued to gain popularity among young consumers [1]. According to the 2018 survey of energy beverage usage among Korean adolescents, consumption has been reported to be almost daily by 3.3%, one to two times a week by 10.9%, one to two times a month by 27.7%, and 58.1% respectively. However, as the grade level of adolescents increases, their drinking rate gradually increases as well among high school students, 5.6% consume EDs daily, 14.7% once a week, 32.4% once a month. Of the total, 52.7% reported drinking energy drinks, higher than the average of all adolescents [2].

The main ingredients of EDs are caffeine, taurine, vitamin B, and sugar [3]. These drinks typically contain more than 80 mg of caffeine per 250 mL, labeled as "high caffeine content." One can of ED represents 70% of the maximum recommended daily intake of caffeine. Caffeine is a commonly used psychostimulant that has a chemical structure similar to that of adenosine, preventing adenosine from binding to the adenosine receptor in the brain [4]. These caffeine actions can constrict blood

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vessels in the brain, and the decrease in brain blood flow contributes to changes in cognitive function [5]. Some EDs contain up to 17 teaspoons of sugar [3]. The consumption of excess sugar is known to have adverse physical and mental effects. Indeed, several components of EDs can cause synergy problems. Increased ED intake and caffeine-related overdose have negative effects on cardiovascular health, such as arrhythmia [6]. Results of studies to mental health as well as the physical effects of EDs have identified associations between ED consumption and mental health issues, such as anxiety and depression symptoms [7]. In addition, studies have shown that the EDs consumed by American college students for psychotropic effects are associated with a low-grade point average (GPA) scores [8].

Adolescence is a transitional period from childhood to adulthood, with changes in physical and mental abilities [9]. Adolescents' brains are structurally and functionally changed and gradually reconstructed. Especially in adolescence, information processing speed and working memory are most noticeably improved [10]. Growing adolescents who consume EDs may show abnormal physical symptoms, such as impaired growth, palpitations, and insomnia [11,12]. In addition, students who consumed EDs show a significantly increased risk for suicidal thoughts compared with those who do not drink EDs [13]. Consumption of high-caffeine EDs in adolescence is associated with alcohol abuse in college [14]. Increased hospitalization and deaths from ED consumption have been reported [15]. However, there have not been many studies on EDs, concentration improvement, and mental health, and most studies have been conducted on adults; research on adolescents is very insufficient [7]. The brain of adolescents undergoes a different developmental process to that of adults, and as such, research results in adults may not apply equally. Moreover, the effects or side effects of EDs may be different from those of adults. EDs are popular among adolescents, but the long- and short-terms effects of chronic use of EDs with high caffeine and various additives are not fully understood.

The present study aimed to identify changes in psychomotor activity, such as anxiety, depression, and cognitive function, by administering EDs to adolescent rats for a long or short time.

METHODS

1. Animals

Male Sprague Dawley rats weighing 180 ± 20 g, 6 weeks old, were purchased through Orient Bio (Seongnam, Korea). The sample size (number of animals) is calculated from the 'resource equation,' and 5 to 7.5 animals per group are needed [16]. However, according to the protocol of the open field test and the forced swim test, which are behavioral tests, 8-20 rats need per group [17,18]. Finally, a total of 27 animals were used, 9 per group. Animals were housed in a place where water and food were freely consumed, and the environment was regulated (22 ± 2°C, the humidity of 50%), and day and night cycle (12 hr light/12 hr dark) controlled conditions. After a week's adaptation period, animals were randomly assigned to the control group (CON), long-term intake group (LT), and short-term intake group (ST).

2. Energy drink application

In this experiment, commercially available energy drinks were used in the research to confirm the overall effect of energy drinks rather than specific ingredients. The drinks stir in room temperature at 100 RPM, to remove the CO_2 contained in the energy drink, and then used to confirm that all air bubbles were removed. According to McConnell (2008), the maximum oral dose in rats is 10 mL - 20 mL/kg [19], and according to a survey by Yoo & Shim (2014), the maximum consumption of energy drinks in Korean university students was two cans per day [20]. However, the half-life of caffeine in humans is 2.5-4.5 hours, whereas rats have a pharmacokinetic difference of 0.7-1.2 hours [21], so more doses should be administered. Therefore, we administered 1.5 mL/100 g of the drink twice a day (9 am/9 pm), which is four cans of human consumption. The long-term administration group (LT) was administered for 14 days, and the short-term administration group (ST) was administered for one day on the day of the behavioral experiment. The control group also received normal saline at the same time and the same method to give the same stress due to oral administration.

3. Behavioral tests

1) Open field test

The Open-field test (OFT) measures activity to determine of anxiety-like behavior. Before the behavioral experiment, the laboratory environment was prepared at a temperature of 23°C, and the lighting slightly dark. Rats are moved to the test site at least 1 hour in advance to adapt to the environment. Rats were placed in a box (77 × 77 × 25 cm), acclimatized for 60 seconds, and observed for 5 minutes. The mobility and activity of the rat were observed by dividing the section into 16 identical

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square lines inside the box. At the end of each experiment, the inside of the box was washed with 70% ethanol. Measure the number of movements from the side and center. It was counted as a movement when all four legs of the rat passed.

2) Forced swim test

Forced swim test (FST) is a behavioral test for rodents developed as a model for predicting the clinical efficacy of antidepressant drugs. Prepare a cylindrical water bottle (100 cm in height and 30 cm in diameter) and fill 2/3 of the water (22 ± 2°C). Each of the Rats was dropped into a transparent cylindrical number. When the animal is first submerged in the tank, the animal's head is gently placed so that it does not fall into the water. The first day was trained for 10 minutes, and the next day conducted the test for 5 minutes. The water in the tank was changed whenever the group changed. After the experiment, the video is analyzed to measure mobility and immobility. The immobility was a moment of floating on the surface of the water without hope without overcoming the stressors. In other words, immobility time means depressing-like behavior.

3) Novel object recognition test

The novel object recognition test (NOR) is used to evaluate cognitive function in animals. This test is a revised version, with habituation, exploration, and new recognition periods, assessed by the difference in search time for new or familiar objects. One day before the test, rats are exposed to the test space for 5-minute habituation. On the first day, two identical objects (A+A) are placed in a test box (60 × 60 × 40) a few centimeters from each other. After 20 seconds of acclimatization time, record the pattern of object search for 10 minutes. On the second day of the test, two different objects (A+B) are placed, where A is the same object used in the familiarization session, and B is the new object (Figure 1A). As in the first test, after 20 seconds of adaptation, rats are probed for 10 minutes to record the object. After the experiment, the video is analyzed to measure the time of interest in each object. Interest was based on the distance ≤ 2 cm between the nose and the object. The difference between groups was analyzed by calculating the discrimination ratio (DR).

$$\text{Discrimination ratio} = \frac{T_N}{T_N+T_F}$$

(T_N: time exploring novel object, T_F: time exploring familiar object)

4) 8-arm radial maze test

An 8-arm radial maze is presented in Figure 1B. It has eight arms, and the labyrinth wall is made of transparent material so that rats can see clues such as the surrounding lab desk, wall corners, and pictures hanging on the wall. Food is placed at the end of each radioactive maze. Rats can visit eight arms to gain food. If the visited arm is revisited, it is set as an error. In addition to learning spatial cues, rats need to remember the arm they visited and the one they didn't. The two types of memory evaluated in this test are reference memory and working memory. In this experiment, the traditional 8-arm radial maze test is modified and used to remove water instead of food. The 8 arm radial maze test was tested in the CON and LT groups to assess spatial learning and memory. Because the ST group was administered only one day on the last day of the behavioral test, it was excluded from this test, which took three days. Remove water from the cage the day before training. On the training day, place 20 μL of water at each end of the 8-arm maze. If the rat is unable to locate the arm within 120 seconds, carefully guide the rat to the nearest

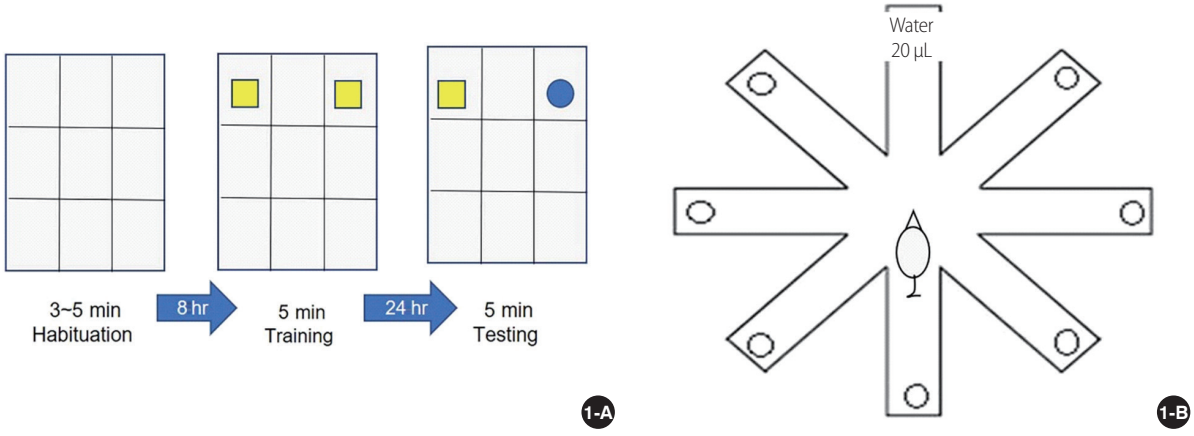

Figure 1. Setting up cognitive function test. The process of novel object recognition test (A), schematic represent of 8-arm radial maze test (B).

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arm for 10 seconds so that it can recognize the target location. After 24 hours, the test is conducted. Place the rat in the central space, enter each of the eight arms, and record the take time and the correct number of times it takes to find water in each of the eight arms. The time limit is 8 minutes. For accurate results, the experiment was repeated three times, and the average value was used.

4. Statistical analysis

Statistical analysis was performed using the SPSS Ver. 25.0 (IBM-SPSS Inc., Chicago, Illinois, USA). All data were shown as the standard error of the mean (SEM) and evaluated for normal distribution by the Shapiro-Wilk test. When the normal distribution and the Levene test are satisfied, the differences of each group were tested using the One-way ANOVA test and the Scheffe's test (Post-hoc). Statistical significance was limited on $p < .05$. If the normal distribution or the Levene test are not satisfied, the group differences were compared using the nonparametric test, the Kruskal-Wallis test. If there is a significant difference between groups after the Kruskal-Wallis test ($p < .05$), the Mann-Whitney test is performed for each group. And then the Bonferroni method (0.05/3) is considered post-hoc ($p < .0167$).

5. Ethical consideration

This study follows the policies of the Animal Experimental Ethics Committee and the laws related to animal experiments and conducted animal experiments in by 3R (replacement, refinement, reduction) principles. All participants in this study completed the training of users of laboratory animal facilities and received appropriate training such as handling animals, administering anesthetics, and sterilizing them. All procedures were conducted after the approval of the study by the Animal Experimentation Ethics Committee of K University (KHSASP-19-227).

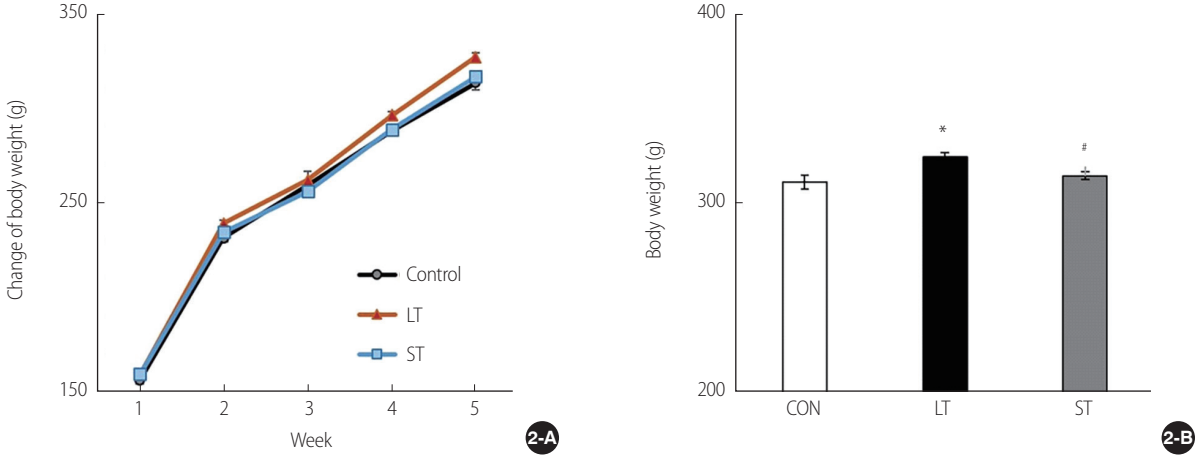

Figure 2. Change of body weight. Weekly body weight gain (A), Body weight after intervention of energy drink (B). Data are expressed as mean ± S.E.
S.E = Standard errors (*$p < .05$ compare to the CON group, #$p < .05$ compare to the LT group); CON = Control group, LT = Long-term intake group; ST = Short-term intake group.

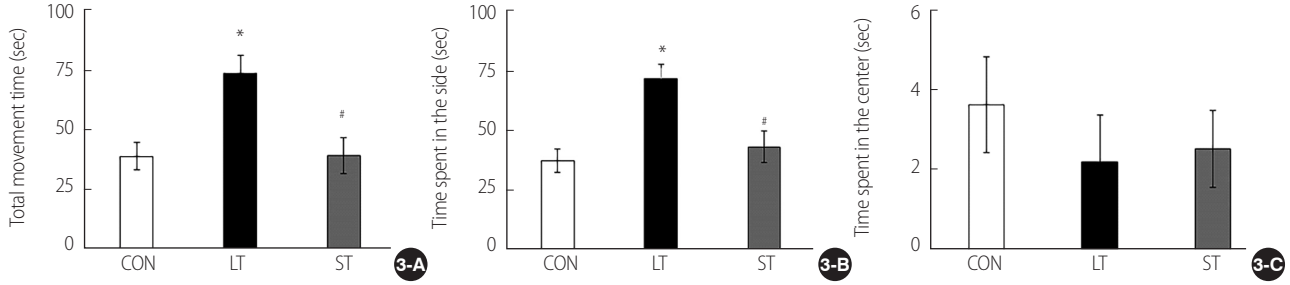

Figure 3. Results of anxiety-like behavior in the open-field test. Total movement time (A), time spent in the side (B), and center (C). Data are expressed as mean ± S.E.
S.E = Standard errors (*$p < .05$ compare to the CON group, #$p < .05$ compare to the LT group); CON = Control group; LT = Long-term intake group; ST = Short-term intake group.

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RESULTS

1. Effects of long- and short-term administration of energy drinks on weight gain

Before the start of the intervention, the mean body weight (g) was 156.22 ± 2.27 in the CON group, 159.33 ± 1.53 in the LT group, and 159.11 ± 1.46 in the ST group, and there was no difference between the control group and the experimental group. (F = 0.94, p = .405) (Figure 2A). After an intervention, body weight (g) was measured as 312.89 ± 3.59 in the CON group, 326.44 ± 1.99 in the LT group, and 316.22 ± 2.12 in the ST group, with significant differences between the groups (F = 7.00, p = .004). As a result of post-hoc analysis, a significant difference occurred between the CON and the LT group (p = .006) and the LT and the ST group (p = .041) (Figure 2B).

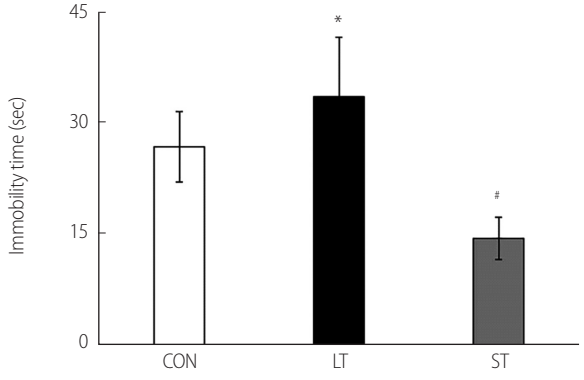

Figure 4. Immobility time in forced swim test. Data are expressed as mean ± S.E.
S.E = Standard errors (*p < .05 compare to the CON group, #p < .05 compare to the LT group); CON = Control group; LT = Long-term intake group; ST = Short-term intake group.

2. Effect of long- and short-term administration of energy drinks on anxiety- and depression-like behavior

In the open field test, an increase of activity of the side region, avoiding exposure in exploratory behavior is an expression of anxiety. The total movement time (sec) was 39.86 ± 5.64 for CON, 74.67 ± 7.64 for LT Group, and 40.14 ± 7.57 for ST Group. It was significantly higher in the LT group compared to the CON group and the ST group (F = 7.81, p = .004) (Figure 3A). Spent of time(sec) in the side region, was measured as a CON group 37.14 ± 4.88, LT group 71.33 ± 6.18, and ST group 43.00 ± 6.51 (F = 9.79, p = .002). Compared to the control group, the movement of the LT group to the side region increased (p = .003), and it was measured higher than the ST group (p = .014) (Figure 3B). However, the time spent in the center area was not different between groups (F = 0.45, p = .642) (Figure 3C).

In the forced swim test (FST), immobility time means learned helplessness and is used as depression-like behavior. Immobility time (sec) was 21.71 ± 3.64 in the control group, 40.59 ± 6.44 in the LT group, and 19.44 ± 2.49 in the ST group (F = 6.51, p = .006). The LT group increased the immobility time compared to the CON group and the ST group (respectively p = .036, p = .011). ST Group was no different from the CON group (Figure 4).

3. Effect of long- and short-term administration of energy drinks on cognitive behavior

Novel object recognition test was conducted to evaluate learning and memory. There were differences between groups (χ^2 = 8.38, p = .015). As a result of the Mann-Whitney test for each group, the LT group was significantly reduced compared to the control group (Z = -2.82, p = .003), but there was no significant difference from the ST group (Z = -2.16,

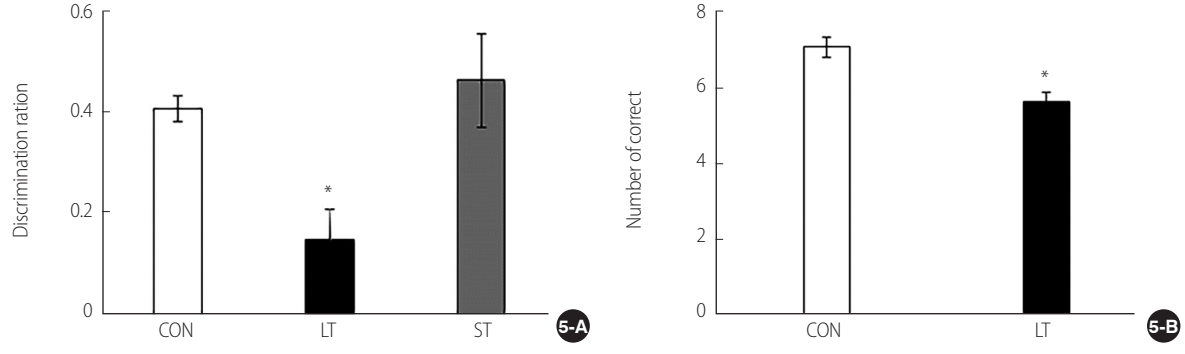

Figure 5. Result of cognition-related behavior test. Discrimination ratio of Novel Object Recognition test (A), Number of correct of 8-arm maze test (B). Data are expressed as mean ± S.E.
S.E = Standard errors (*p < .017 compare to the CON group); CON = Control group; LT = Long-term intake group; ST = Short-term intake group.

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$p = .029$) (Figure 5A).

An 8-arm radial maze test was performed to evaluate learning and memory between the control and LT groups. The number of correct was 7.11 ± 0.26 for the CON group and 5.67 ± 0.24 for the LT group, which significantly reduced the spatial cognitive function in the long-term administration group of energy drinks ($Z = -2.98$, $p = .003$) (Figure 5B).

DISCUSSION

This study highlighted the behavioral changes caused by long-term ED consumption in adolescence. The long-term intake EDs increased anxiety- and depression-like behavior, as well as decreased learning and memory function. In the long-term administration group (LT) showed a significantly increased body weight compared with the control and short-term administration (ST) groups. EDs contain plenty of sugar and caffeine [22]. In our study, the amount of ED consume by the animals was 3 mL per day, which contained 1.1 mg of caffeine and 456 mg of sugar. In terms of human consumption, their consumption is equivalent to four cans, and the sugar content would be 152 g (56 sugar cubes). Park et al. (2019) reported that long-term administration of high-level caffeine is not associated with weight gain [23], although another study suggested that caffeine consumption may reduce insulin sensitivity [24]. Rush et al. (2006) confirmed that the combination of sugar and caffeine in EDs can contribute to obesity by promoting insulin resistance and fat production through increasing carbohydrate oxidation and reducing lipid oxidation [25]. As such, the present results are in line with those in previous studies. However, it is difficult to determine the risk of obesity in EDs based on the results of this study.

In the OFT and FST of this study, the ST group did not show reduced anxiety, depression, or motility. This result is contrary to a previous finding that EDs increase physical performance in exercise [26]. In the LT group, the activity increased by 1.87 times compared with the control group. This difference is thought to be owing to the 1.92-fold increase in the time in the side region. Notably, the LT group showed increased anxiety- and depression-like behaviors. Studies related to mental health and EDs have conflicting findings. Results have shown that EDs have a positive effect on mood, vitality, and social extroversion [7]. Meanwhile, other studies have reported an increase in tension and anxiety scores as an adverse effect [27]. The previously reported improvement effect of EDs on attention seems to be temporary, and a direct comparison is not possible given that the study experimented with human adults. Chronic use of EDs drinks has been reported to be sufficiently related to anxiety, depression, self-mutilation, and suicidal thoughts [28]. The results of our study also provide consistent results.

In the two cognitive-behavioral tests in this study, the LT group had significantly reduced spatial learning and memory function compared with the control group. In particular, the eight-arm radial maze allowed us to evaluate the reference memory (when entering the arm to find compensation) and working memory (when entering the other arm from the arm previously entered). The decrease in the number of correct attempts in the LT group suggested that the long-term consumption of EDs can interfere with learning and memory. According to previous studies the higher the energy drink consumption of students, the lower their grade point average (GPA) score [29]. Our findings support the results of previous studies.

The results of the four behavioral tests showed that long-term administration of EDs increases behavior such as anxiety and depression, resulting in reduced memory owing to decreased concentration. Histological analysis after three weeks of ED administration in a similar experimental design to this study showed a reduction of neurotransmitters, such as NE, DA, and GABA in the hippocampus of rats [30]. However, this previous study did not perform behavioral tests, so it is difficult to explain causal relations with respect to the current study. Other studies have shown that high sugar intake leads to brain inflammation and memory impairment [31]. Additionally, high concentrations of caffeine have been reported to cause short-term memory impairment in adolescent rats [32]. Therefore, the effect of EDs on reducing cognitive function may be attributed to the sugar or caffeine content. This study did not independently test the components, it was not possible to determine whether the decline in cognitive function was due to the high concentrations of sugar or caffeine. Nevertheless, the current results are relevant because most EDs contain high levels of both caffeine and sugar.

An important implication of this study is that the long-term consumption of EDs using laboratory animals showed increases in anxiety- and depression-like behaviors, and also decreases in learning and memory. In animal experiments, strict environmental control is possible, and behavior tests can be applied in the same way, thereby enabling the production of meaningful data and evidence. Moreover, long-term application for 14 days in rats makes more sense because it corresponds to one year in human time. However, to reveal the overall long- and short-term

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effects, analysis of each ingredient and component combination is required. This is a limitation of the present study. Future research should investigate the effects of individual components and concentrations. Despite evidence that the consumption of EDs is associated with adolescents' health and risky behavior, EDs continue to be popular with young consumers. Therefore, more research is needed in the future.

CONCLUSION

Anxiety- and depression-like behaviors increased, and the function of learning and memory decreased in adolescent rats that consumed EDs for a long time. Nurses and health care providers should be knowledgeable about the risk of ED consumption in adolescents to provide proper education and conduct appropriate practice.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHORSHIP

LJH, CYJ, and LJH contributed to the conception and design of this study and performed the collecting data, the statistical analysis and interpretation; LJH and KYJ drafted the manuscript and critically revised the manuscript; KYJ supervised the whole study process. All authors read and approved the final manuscript.

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REFERENCES

1. Lim HO. Survey on safety of energy drink. Trend report. Seoul: Korea Consumer Agency; 2013.
2. Ministry of gender equality and family. Frequency of use of high caffeine (energy) drinks [Internet]. Seoul: Statistic Korea: 2018. [cited 2019 Nov 04] Available from: http://kosis.kr/statHtml/statHtml.do?orgId=154&tblId=DT_MOGE_15400027 21&conn_path=I2
3. Higgins JP, Tuttle TD, Higgins CL. Energy beverages: content and safety. Mayo Clinic Proceedings. 2010;85(11):1033-1041. https://doi.org/10.4065/mcp.2010.0381
4. Wassef B, Kohansieh M, Makaryus AN. Effects of energy drinks on the cardiovascular system. Journal of World Cardiology. 2017;9(11):796-806. https://doi.org/10.4330%2Fwjc.v9.i11.796
5. Lunt MJ, Ragab S, Birch AA, Schley D, Jenkinson DF. Comparison of caffeine-induced changes in cerebral blood flow and middle cerebral artery blood velocity shows that caffeine reduces middle cerebral artery diameter. Physiological Measurement. 2004;25(2):467-474. https://doi.org/10.1088/0967-3334/25/2/006
6. Mangi MA, Rehman H, Rafique M, Illovsky M. Energy drinks and the risk of cardiovascular disease: a review of current literature. Cureus. 2017;9(6):e1322. https://doi.org/10.7759%2Fcureus.1322
7. Richards G, Smith AP. A review of energy drinks and mental health, with a focus on stress, anxiety, and depression. Journal of Caffeine Research. 2016;6(2): 49-63. https://doi.org/10.1089%2Fjcr.2015.0033
8. Buchanan JK. Energy drink consumption (with and without alcohol) and its relationship to risky behavior, risk awareness, and behavioral intention in college students [Dissertation]. Kentucky: University of Kentucky; 2012. p. 37-55
9. Giedd JN. The teen brain: insights from neuroimaging. Journal of Adolescent Health. 2008;42(4): 335-343. https://doi.org/10.1016/j.jadohealth.2008.01.007
10. Gathercole SE, Pickering SJ, Ambridge B, Wearing H. The structure of working memory from 4 to 15 years of age. Developmental Psychology. 2004;40(2):177-190. https://doi.org/10.1037/0012-1649.40.2.177
11. Reissig CJ, Strain EC, Griffiths RR. Caffeinated energy drinks—a growing problem. Drug and Alcohol Dependence. 2009;99(1):1-10. https://doi.org/10.1016/j.drugalcdep.2008.08.001
12. Lee SJ, Kim HC, Kim MR. Analysis on intake of energy drinks of high school students in Gyeoungbuk region. The East Asian Society of Dietary Life. 2014;24(6):924-932. https://doi.org/10.17495/easdl.2014.12.24.6.924
13. Park JH, Hahm MI, Kim SJ, Min IS. Association between high-caffeine energy drink intake and suicidal ideation in Korean adolescents. Journal of the Korean Society of School Health. 2016;29(2): 71-80. https://doi.org/10.15434/kssh.2016.29.2.71
14. Skewes MC, Decou CR, Gonzalez VM. Energy drink use, problem drinking and drinking motives in a diverse sample of Alaskan college students. International Journal of Circumpolar Health. 2013;72(1):1-6. https://doi.org/10.3402/ijch.v72i0.21204
15. Mattson ME. Update on emergency department visits involving energy drinks: a continuing public health concern [Internet]. Rockville (MD): Substance Abuse and Mental Health Services Administration (US); 2013[cited 2020 Apr 24]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK384664/ PMID: 27606410
16. Charan J, Kantharia ND. How to calculate sample size in animal studies? Journal of Pharmacy & Pharmacotherapeutics. 2013;4(4): 303-306. https:// doi.org/10.4103/0976-500X.1197266
17. Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. Journal of Visualized Experiments. 2015;(96):e52434. https://doi org/10.3791/52434
18. Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity in rodents. Nature Protocols. 2012;7(6): 1009-1014. https://doi.org/10.1038/nprot.2012.044
19. McConnell EL, Basit AW, Murdan S. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in vivo experiments. The Journal of Pharmacy and Pharmacology. 2008;60(1):63-70. https://doi.

org/10.1211/jpp.60.1.0008
20. Yoo HS, Sim KH. Survey on the high-caffeine energy drink consumption status of university students in Seoul. The East Asian Society of Dietary Life. 2014;24(3):407-420. https://doi.org/10.17495 /easdl.2014.06.24.3.407
21. Nehlig A. Are we dependent upon coffee and caffeine? A review on human and animal data. Neuroscience & Biobehavioral Reviews. 1999;23(4):563-576. https://doi.org/10.1016/S0149-7634%2898%2900050-5
22. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. The American Journal of Clinical Nutrition. 2006; 84(2):274-288. https://doi.org/10.1093/ajcn/84.1.274
23. Park JM, Kim Y, Kim H, Kim YJ. Influence of short-and long-term high-dose caffeine administration on behavior in an animal model of adolescence. Journal of Korean Biological Nursing Science. 2019;21(3):217-223. https://doi.org/10.7586/jkbns.2019.21.3.217
24. Keijzers GB, De Galan BE, Tack CJ, Smits P. Caffeine can decrease insulin sensitivity in humans. Diabetes Care. 2002;25(2): 364-369. https://doi.org/10.2337/diacare.25.2.364
25. Rush E, Schulz S, Obolonkin V, Simmons D, Plank L. Are energy drinks contributing to the obesity epidemic? Asia Pacific Journal of Clinical Nutrition. 2006;15(2): 242-244.
26. Lara B, Gonzalez-Millán C, Salinero JJ, Abian-Vicen J, Areces F, Barbero-Alvare JC, et al. Caffeine-containing energy drink improves physical performance in female soccer players. Amino Acids. 2014;46(5):1385-1392. https://doi.org/10.1007/s00726-014-1709-z
27. Wesnes KA, Barrett ML, Udani JK. An evaluation of the cognitive and mood effects of an energy shot over a 6 h period in volunteers. A randomized, double-blind, placebo controlled, cross-over study. Appetite. 2013;67:105-113. https://doi.org/10.1016/j.appet.2013.04.005
28. Salinero JJ, Lara B, Abian-Vicen J, Gonzalez-Millán C, Areces F, Gallo-Salazar C, et al. The use of energy drinks in sport: perceived ergogenicity and side effects in male and female athletes. British Journal of Nutrition. 2014; 112(9):1494-1502. https://doi.org/10.1017/S0007114514002189
29. Champlin SE, Pasch KE, Perry CL. Is the consumption of energy drinks associated with academic achievement among college students? Journal of Primary Prevention. 2016;37(4):345-359. https://doi.org/10.1007/s10935-016-0437-4
30. Bawazir AE. Effects of Energy Drink (Red bull) on some neurotransmitters content and histological structure in the hippocampus region in male albino rats. International Journal of Pharmaceutical Research & Allied Sciences. 2017; 6(2):263-276.
31. Beilharz JE, Maniam J, Morris MJ. Short-term exposure to a diet high in fat and sugar, or liquid sugar, selectively impairs hippocampal-dependent memory, with differential impacts on inflammation. Behavioural Brain Research. 2016;306: 1-7. https://doi.org/10.1016/j.bbr.2016.03.018
32. Jacques A, Chaaya N, Beecher K, Ali SA, Belmer A, Bartlett S. The impact of sugar consumption on stress driven, emotional and addictive behaviors. Neuroscience & Biobehavioral Reviews. 2019;103:178-199. https://doi.org/10.1016/j.neubiorev.2019.05.021