Original

Fearless Energy Drink Potentiates Anxiety, Oxidative Stress, and Neuronal Cell Damage on the Hippocampus of Adult Male Wistar Rat

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Article history: Received 11 November 2023, Reviewed 19 December 2023, Accepted for publication 23 December 2023

How to cite this article:
Nwakanma AA & Idaguko CA. Fearless Energy Drink Potentiates Anxiety, Oxidative Stress, and Neuronal Cell Damage on the Hippocampus of Adult Male Wistar Rat. The Nigerian Health Journal 2023; 23(4):926 - 934. DOI: https://www.doi.org/10.60787/tnhj-746

Abstract

Background: The relationship between the impact of Fearless energy drinks on the hippocampus has not yet been documented. Study investigated the effect of Fearless energy drinks on oxidative stress and hippocampal microstructure in Wistar rats.

Method: Twenty male rats weighing 160-200g were divided into four groups of five rats each. Group A was the control, groups B, C and D received Fearless energy drink orally using gastric tube with the following doses: 2.5ml, 5ml and 7.5ml/kg/day respectively for three weeks. At the end, elevated plus maze test was carried out to check for anxiety. The animals were sacrificed and oxidative stress markers such as superoxide dismutase (SOD) and malondialdehyde (MDA) were carried out; the brains were dissected for hippocampal specimens, which were processed for histological studies using hematoxylin and eosin method.

Result: There was an increased level of anxiety-like behavour in animals in the experimental groups, as they spent more time in the close arm of the maze than in the open arm of the maze when compared to the control. There was a significant decrease ($p \leq 0.05$) in SOD and a significant increase ($p \leq 0.05$) in MDA, which indicate oxidative stress. Histological examination of the hippocampus from Fearless EDs groups showed vacuolation, hypertrophied pyramidal cells with numerous pyknotic nuclei, cracks in pyramidal cell layer and decreased cellular density in the pyramidal cell layer when compared to the control.

Conclusion: Fearless energy drinks administration induced anxiety, deficient antioxidant capacity and morphological change on the hippocampus of Wistar rats.

Keywords: Neurobehavour, hippocampus, fearless, energy drinks, elevated plus maze, anxiety, oxidative stress

Introduction

Energy drinks (EDs) are carbonated drinks that include stimulating ingredients, plus there are numerous brands and different types of energy drink; and they are readily available worldwide.[1] The majority of energy drinks contain ingredients that give the body a lot of mental and physical energy, including guarana (a plant product containing concentrated caffeine), taurine, creatine, glucuronolactone, sugar, sodium, and herbal supplements like ginseng, ginkgo biloba, and many

Print ISSN: 0189-9287 Online ISSN: 2992-345X

vitamins.[2] Energy drinks mostly contain caffeine (1,3,7-trimetilxantine), which has psychoactive properties. It is typically present in high concentrations, which frequently causes overconsumption and harmful effects.[2,3]

Though caffeine is quickly and completely absorbed when taken orally and has an elimination half-life of around 4 to 5 hours.[4] It relates to benefits in cognitive capacities, such as memory and concentration. Caffeine functions as a psychostimulant in both humans and rats by boosting alertness, lowering anxiety, and enhancing restfulness.[5] These beverages are widely ingested by young people, athletes, and college students to boost their energy levels and make up for sleep deprivation; they may also enhance mood, physical performance, and lessen mental tiredness.[6] But according to a study, the majority of college students who consume energy drinks (64.3%) are unaware of their potential negative effects, which include anxiety, insomnia, gastrointestinal disorders and tachycardia.[7]

Additionally, the brain undergoes a phase of maturation during adolescence that necessitates adjustments to its neurotransmission and synaptic plasticity, as well as structural alterations in particular areas (such as the hippocampus, limbic system and prefrontal cortex).[8] Concerns about the effectiveness and healthfulness of energy drinks were recently raised by the International Society of Sports Nutrition.[9] The consumption of energy drinks has also been linked to an alarming number of cardiovascular side effects, including arrhythmia, cardiac arrest, and even sudden death.[10,11] Additionally, a number of authors claimed that drinking caffeinated energy drinks could cause anxiety, stress, hyperactivity, headaches, and exhaustion in the brain.[2,12] Others reported more negative side effects, such as sadness, disturbed sleep, and irritability.[13,14]

The limbic system includes the hippocampus, which is crucial for learning, emotions, and consolidating recent short-term memories into long-term memory.[15,16] The cortical and subcortical networks that make up the limbic system are interrelated, and they are in charge of tying visceral feelings and emotions to cognition and behavior.[17] The Cornu Ammonis and the dentate gyrus (DG) are the two gray matter laminae that make up the hippocampus. The four regions of the Cornu Ammonis are designated as CAl, CA2, CA3, and CA4.[18] The hippocampal cornu ammonis 1 (CA1, CA3, and DG) is the first area of the brain to experience a reduction in function[19] and is thought to be the region with the greatest vulnerability that is susceptible to numerous stressors, including oxidative damage.[20] Another delicate brain region, the dentate gyrus (DG), contains adult neural stem cells that are in charge of adult hippocampal neurogenesis.[21] Consuming EDs may cause oxidative stress and an inflammatory response in the brain, according to recent research.[22,23] When the body's defense mechanisms against oxidative stress and the production of oxidant chemicals are out of balance, oxidative stress results. Reactive oxygen species (ROS) and free radicals are often created continuously at low levels as a result of typical and vital biological processes.[24] When they are created in excess, they can harm or even kill cells, therefore, resulting in damage to lipids, proteins, and nucleic acids.[25]

The brain is very susceptible to the harmful effects of free radicals and reactive species owing to its high use of oxygen, rich lipid content, and low amounts of antioxidant enzymes in comparison to other tissues.[26] A reactive astrogliosis and a rise in pro-inflammatory cytokines, which result in neuronal death, control these mechanisms.[22] The elevated plus maze (EPM) test is a commonly used behavioral experiment in neuroscience to measure mice' anxiety-related behavior. Without conditioning the animals first, it can be automated to produce reliable findings in a 5-minute test.[27] Indicators of open space-induced anxiety in mice include the number of times they enter the maze's open arms and the amount of time they stay there.[28] However, research on how Fearless energy drinks affect anxiety and hippocampus formation is still lacking. We intended to assess the effects of Fearless energy drinks on anxiety and oxidative stress due to their widespread excessive consumption and the fact that their toxicological concerns are mostly unknown. The purpose of the current study is to determine how ingestion of the Fearless energy drink affects adult male Wistar rats' anxiety-related behaviors, oxidative stress indicators, and hippocampal histology.

Method

Chemical

Fearless energy drink was purchased from main market in Onitsha, Anambra State. Nigeria. The ingredients contained in the energy drinks (fearless) were as follows: Water, Sugar, citric acid E 330, Carbon dioxide, Acidity Regulator Sodium Citrates E 331, Preservatives Potassium Sorbate E 202 and Sodium Benzoate E 211, flavouring, Caffeine, Taurine, Niacin, Inositol, Colours

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Tartrazine E 102 and Sunset Yellow FCF E 110, Vitamin B6, Vitamin B12 and Ginseng Extract.

Procurement of animals

Twenty adults male wistar rats (Rattus norvegius) weighing between the ranges of 160-200g, were obtained from Iyke Animal Farm, located at Nnewi Anambra State. The animals were housed in the experimental house of the Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus. Anambra State. The animals were fed on standard rat diet and allowed free water access. Animals were allowed to acclimatize to experimental conditions by housing them for 14 days prior to experiment.

Experimental protocol

Ethical approval for the study was obtained from Faculty of Basic Medical Sciences Ethical Committee, with ethical approval number COOU/FBMS/005 and the experiment complied with the National Institution of Health Guideline Principles of Laboratory Animal in Biomedical Research.[29]

Twenty adult male rats were equally and randomly divided into four groups of five rats each.
Group A (Control group): rats left untreated had access to distilled water and feed only.
Group B: rats received Fearless at dose of 2.5 ml/kg/ bw/day
Group C: rats received Fearless at dose of 5.0 ml/kg/ bw/day
Group D: rats received Fearless at dose of 7.5 ml/kg/ bw/day
The administration of energy drink was done orally using gastric tube for three weeks

Elevated Plus maze

Rat models were utilized to evaluate anxiety-related behavior using the elevated plus maze test. It establishes a preference between a risky setting (the raised open space) and one that is relatively safe and comfortable (the closed arm). Two open, perpendicular arms (50 x 10 x 50 cm) are crossed at the center platform (10 x 10 cm) to form the plus sign in the elevated plus maze. The entire device was elevated 5 cm off the ground. Starting from the middle platform, the rats approached the open arm. The rats were allowed to explore the maze for five minutes. The measurements of anxiety-like behavior include the number of entries into the open arm compared to the total number of entries, and the amount of time spent on the open arms compared to the closed arms. As a result, both the number of entries and the length of time spent in each arm were recorded. The animal is said to be in each arm when the four limbs are in the arm. After each session, the maze was cleaned with ethanol to avoid olfactory cues of the rats.

Sample collection

At the end of the experiment, the rats were fasted for 12 hours and blood were collected from retro orbital veins into plain tubes and preserved at room temperature for 15 min, then centrifuged for 10 min at 3000 Xg to get serum. Serum aspirated, aliquot and kept frozen at (-20ºC) until usage. Oxidative stress markers such as superoxide dismutase (SOD) and malondialdehyde (MDA) were carried out in Central Laboratory by commercially available kits according to kits protocol.

Histology examination

Following blood withdrawal, rats of all groups were euthanized by cervical dislocation under ether anesthesia, the brain were excised and hippocampus removed and fixed in 10% buffered formalin solution. The hippocampus were processed for paraffin blocks. Serial sections of 5 micron thick were done. The sections were stained with hematoxylin and eosin (H&E).

Statistical Analysis

The data obtained during the study were analyzed using Graphpad Prism Statistics version 8.0.1. Collected values were presented as mean ± SD. Statistical comparisons between groups were made by One-way analysis of variance (ANOVA) followed by Bonferroni's (post-hoc test), $P < 0.05$ was considered statistically significant.

Results

Effect on anxiety

Reports showed that rats administered Fearless energy drink (groups B, C and D) stayed longer in the close arm than the open arm of the maze, when compared to the control group A. The control group tends to explore both arm of the maze simultaneously. Fig 1A and 1B:

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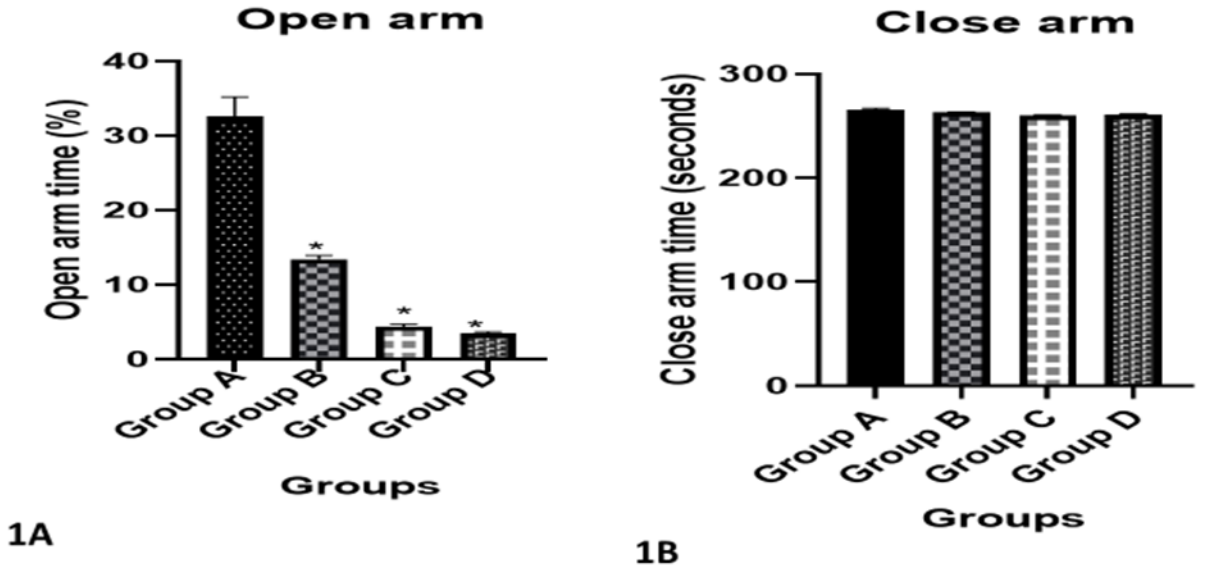

Figure 1A and 1B: Values are represented as mean ± SD. significant level of difference at *p < 0.05 when compared to the control

Effect on oxidative stress

Fearless energy drink treated animals showed significant decrease ($p \leq 0.05$) in the level of SOD, when compared to control group (Fig 2A). There was also a significant increase ($p \leq 0.05$) in MDA, which was dose dependent when compared to the control group Fig 2B.

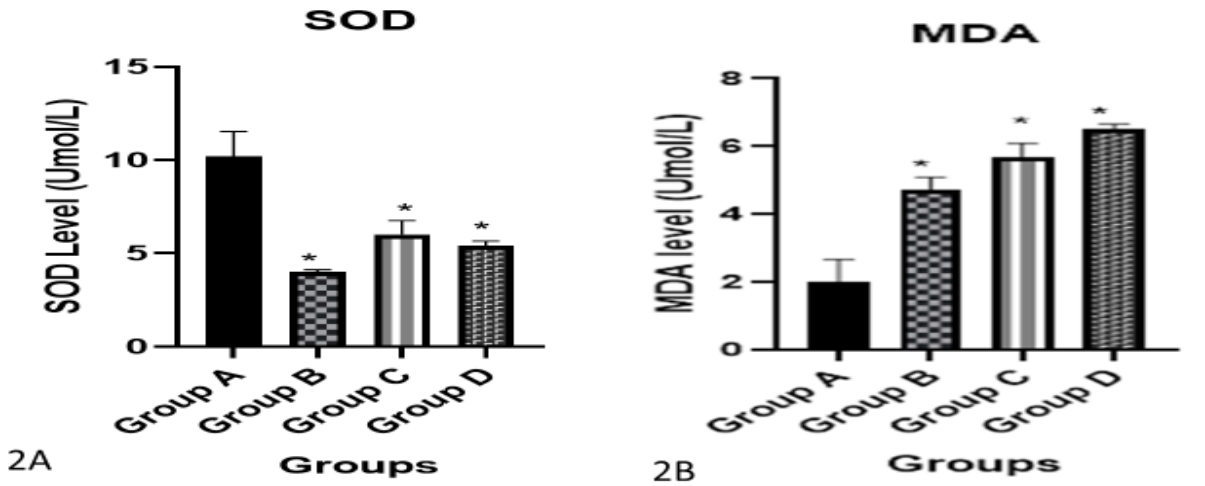

Figure 2: Analysis of SOD and MDA activity levels of Fearless energy drink treated rats. Values are represented as mean ± SD. significant level of difference at *p < 0.05 when compared to the control (group A)

Effect on neuronal cell damage

Histological Results

The hippocampus of the control group A showed the three cell layers, molecular (M), pyramidal (PY) and polymorphic (PM) layers. Molecular layer has dendrites

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and neuronal axons. The pyramidal layer has pyramidal cells, axons, dendrites and vesicular nuclei while the polymorphic layer consists of axons, dendrites, scattered nerve cells and glia cells. Fearless ED groups, histological examination of the hippocampus showed vacuolation, hypertrophied pyramidal cells with numerous pyknotic nuclei and cracks in pyramidal cell layer and decreased cellular density of pyramidal cell layer when compared to the control group with normal histoarchitecture.

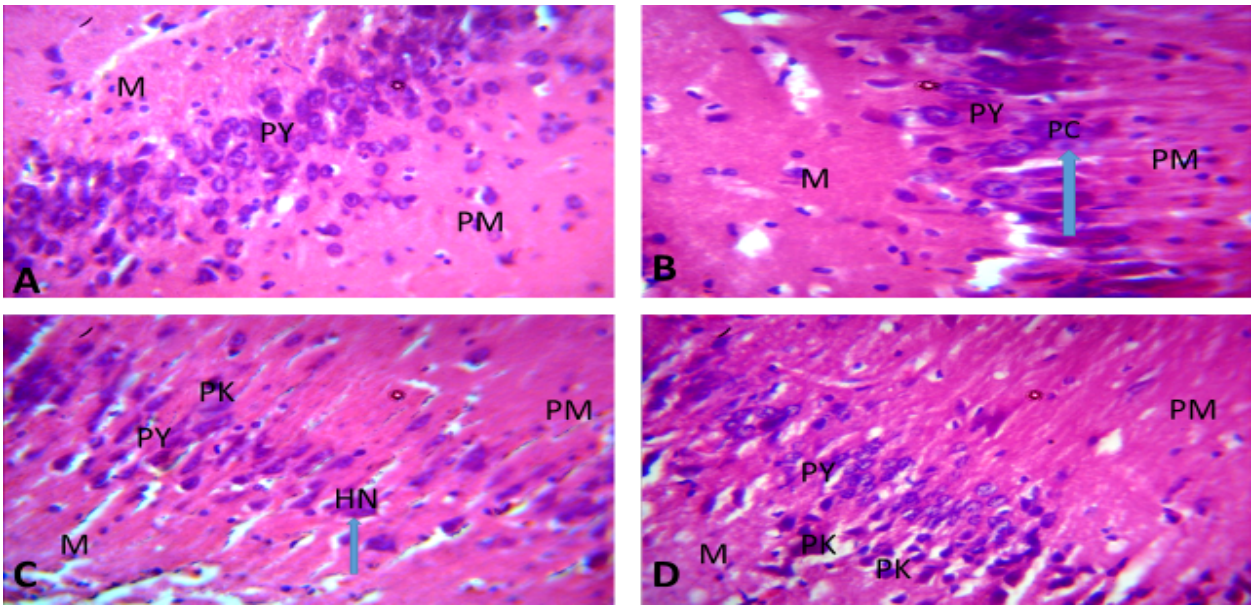

Figure 3: Photomicrograph of Groups A (control section) showing hippocampus with distinct molecular (M), pyramidal (PY) and polymorphic (PM) layers. Group B administered 2.5ml of Fearless energy drink showing hypertrophied pyramidal cells (PC) (arrow). Group C administered 5ml of Fearless energy drink showing pyknotic (pk) and hypertrophied nuclei (HN) (arrow). Group D administered 7.5ml of Fearless energy drink showing numerous pyknotic nuclei in the pyramidal layer. (x400 H/E)

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Discussion

This study examined the effects of Fearless energy drink use on the hippocampus, anxiety, and antioxidant levels in rats. When compared to the control group, the results showed that the levels of SOD were much lower in the groups treated with the Fearless energy drink. This study's observation of a decrease in serum SOD levels was consistent with those of Ekaluo et al,[30] who found that caffeine decreases the antioxidant defense system, including SOD, glutathione peroxidase (GPx), and catalase (CAT), which then causes an increase in free radical activities and oxidative stress. To prevent cells from being oxidatively destroyed by reactive oxygen species (ROS), these enzymes serve as the body's main antioxidants. They work in conjunction with non-enzymatic antioxidant pathways. SOD converts excess reactive superoxide anion to hydrogen peroxide in order to neutralize it.[31] Additionally, MDA levels considerably ($p<0.05$) increased in mice given the Fearless energy drink, indicating lipid peroxidation activity, an important biological effect of oxidative cellular damage. As a result, the rise in MDA levels is a reflection of oxidative stress. This finding is in line with information from studies of energy drinks given to rats, which revealed a significant drop in antioxidant levels.[32,33]

Recent research has shown how ED contributes to oxidative stress in various organs, increased reactive oxygen species (ROS) generation, and decreased antioxidant defenses.[23] A normal ED has roughly 320 mg/Kg of caffeine, which is well recognized to be harmful to human health when consumed in excess and over an extended period of time.[2,34,35] Other components in Fearless may also be harmful to human health. The majority of the evidence suggests that the health risks associated with EDs are caused by their high quantities of sugar and caffeine.[36]

Significant predictive accuracy exists for the raised plus maze test when screening for anxiolytic drugs;[28] the number of entries into the open arms and the time spent there are notably increased by anxiolytic substances and decreased by anxiogenic compounds. A useful indication of overall activity is thought to be the combined score and distance of all entries. The number of entries is another measure of anxiety, and the proportion of entries and the amount of time spent in each arm make up the principal anxiety index.[37] The avoidance of the open arms is thought to occur from the induction of higher levels of fear since the open and closed arms are thought to stimulate the same exploration desire.[38] The fear of elevated and open spaces is assumed to be the reason why mice refuse to enter the maze's open arms.[28] When it comes to anxiety, animals given Fearless energy drink had higher levels of anxiety because they spend more time in the close arm of the maze than the control group, which simultaneously explores both arms. Energy drinks mostly contain caffeine and sugar content, which is well known as a stimulant of the central nervous system that activates noradrenaline and serotonin neurons.[39,40] Energy drinks with caffeine may also activate methylxanthine, which has been linked to psychiatric conditions like anxiety.[39,41]

Caffeine is an example of an anxiety-inducing drug.[42] Caffeine administration to mice, whether acute or chronic, has been shown to elicit effects similar to anxiety.[43,44] The main harmful effect of EDs has also been attributed to excessive caffeine concentrations.[45] However, it was impossible to pinpoint a specific component of EDs that caused injury to the nervous system.[23] According to reports, consuming caffeine, taurine and guarana together may cause cell death via lowering antioxidant activity;[46] hence, this can results in the hippocampus rapidly losing its function, since it is vulnerable to oxidative stress and other insults.[19]

In addition, when compared to the control group, which had normal cytoarchitecture, the hippocampus from the fearless EDs groups showed histological changes in the form of pyknosis, vacuolation, and hypertrophied pyramidal cells. Caffeine, which is the primary ingredient in energy drinks, has been shown to negatively affect synaptic plasticity in hippocampal neurons by inhibiting long-term potentiation at the synapses, which could explain these effects.[47] This study showed that consuming the Fearless energy drink could increase oxidative stress. The effects of caffeine in combination with the other substances may be to blame for the overall effect shown in this study.

Strength and limitation of the study

The consumption of Fearless energy drinks is generally on the increase among youths possibly because of its pleasant taste, stimulant effect, availability and affordable price. The study's limitations may include the short duration of the study; which may not be enough to reveal the changes in the hippocampus. Further studies need to corroborate these observations in humans.

Implications of the findings of the study

The findings of this suggests that Fearless energy drink may cause microanatomical damage to the hippocampus.

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Conclusion

The current study demonstrated the detrimental effects of Fearless energy drink on oxidative stress, anxiety, and the histological structure of the hippocampus. These outcomes could be directly linked to the timing and dose-dependent consumption of such a drink.

Declarations

Ethical consideration: Ethical approval for the study was obtained from Faculty of Basic Medical Sciences Ethical Committee, with ethical approval number COOU/FBMS/005 and the experiment complied with the National Institution of Health Guideline Principles of Laboratory Animal in Biomedical Research.[29]

Authors' contribution:
The authors were involved at different stages of writing the manuscript.
Conception and design of the research: Akudo NA and Idaguko CA.
Conduct of the research: Akudo NA
Analysis and interpretation of data: Akudo NA and Idaguko CA.

Conflict of interest: The authors declare no competing interest.

Funding: No funding was received to assist with the preparation of this manuscript.

Acknowledgement: The authors would like to express our special thanks to Dr. Chukwuebuka Elemuo for the technical assistance rendered during the research.

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