

Implication of Some Energy Drink Mixture with Flunitrazepam on Endurance Pattern and Cognitive/Motor Functions in Wistar rats.

ABSTRACT

This study was conducted to investigate the effects of some energy drinks mixture with Flunitrazepam on endurance pattern and cognitomotor activities in male Wistar rats. 45 Wistar rats were divided into 8 groups. Group 1 received distilled water; Group 2 received energy drink (A) (3.75 mg/kg). Group 3 energy drink (A) (7.5 mg/kg) Group 4 received energy drink (B) (3.75 mg/kg). Group 5 energy drink (B) (7.5 mg/kg) Group 6 received flunitrazepam (0.03 ml/kg), Group 7 received 3.75 ml/kg of energy drink (A) and 0.03 ml/kg of flunitrazepam, and Group 8 received 3.75 ml/kg of energy drink (B) and 0.03 ml/kg of flunitrazepam. Administration of the mixture lasted for 28 days while endurance test/Cognitive and motor functions test were conducted weekly using Handgrip test, beam walk test and navigational maze test. The result reveals significant improvement in endurance pattern and cognitomotor functions in groups administered with energy drinks alone at week one, however the mixture of energy drink with various doses of Flunitrazepam showed significant impairment in both endurance pattern, and cognitomotor activities. It was therefore concluded that while the combination of energy drinks and flunitrazepam may offer short-term benefits in terms of endurance, and alertness, there is a dose and time dependent significant decline in cognitive and motor functions in wistar rats. The findings suggest significant variations in endurance and cognitive-motor performance across different groups, highlighting the complex interactions between stimulant and sedative substances and their implications for health and behavior.

Keywords: Energy drinks, Flunitrazepam (Rophinol), Cognition, motor functions, endurance.

1.0 Introduction

Energy drinks have gained popularity for their stimulant properties, containing ingredients like caffeine and taurine that are marketed to enhance alertness and physical performance [1]. However, concerns arise when these beverages are combined with sedative-hypnotic drugs like Flunitrazepam, known for their central nervous system depressant effects and potential cognitive impairments [2]. This combination may lead to complex interactions affecting both physical

endurance and cognitive-motor activities, highlighting the need for further research in this area [3].

Drug abuse is a pressing public health issue, with the misuse and mixing of substances posing significant risks to individuals' health. The combination of energy drinks with sedative-hypnotic drugs, such as Flunitrazepam, raises concerns in the context of substance abuse [4]. This combination may be used recreationally to intensify the effects of the sedative-hypnotic drug or counteract sedation with the stimulant properties of the energy drink, leading to unpredictable and potentially dangerous outcomes [5]. Moreover, the masking of sedative effects by stimulants in energy drinks may create a false sense of alertness, potentially contributing to continued substance misuse and dependency [6]. Understanding the motivations behind combining energy drinks with sedative-hypnotic drugs in the context of drug abuse is crucial for developing effective prevention and intervention strategies. By investigating the effects of specific energy drink mixtures combined with Flunitrazepam on endurance patterns and cognitomotor activities in male Wistar rats, this study aims to provide insights into the potential risks associated with this combination and contribute to a better understanding of the impact of mixing these substances on physical and cognitive functions.

Materials and Methods

In this research, a group of 45 rats, each weighing an average of 125 grams, was used. The rats were obtained from the animal facility at the Faculty of Basic Medical Science, University of Port Harcourt. They were housed in a controlled environment with standard room temperature and given standard finisher feeds (Top feed, Nigeria) along with water for a two-week

acclimatization period prior to the start of the experimental protocols. The animals were then separated into groups according to the experimental design below:

SN	GROUP NAME	SUBSTANCE ADMINISTERED
1	Group 1(Control)	Normal feed and water
2	Group 2	Energy drink (A) low Dose (3.75ml/kg)
3	Group 3	Energy drink (A) high Dose (7.5ml/kg)
4	Group 4	Energy drink (B) drink low dose (3.75ml/kg)
5	Group 5	Energy drink (B) high dose (7.5ml/kg)
6	Group 6	Flunitrazepam (0.003mg/kg)
7	Group 7	Energy drink (A) low dose + Flunitrazepam (0.003mg/kg)
8	Group 8	Energy drink (B) low dose + Flunitrazepam (0.003mg/kg)

List 1. Experimental design

Administration of various doses of test substances was carried out through oral route and lasted for 28 days. Following administration, various motor and cognitive tests were carried out to determine endurance pattern and cognitive function test in the rat model.

Tests of Endurance and Cognition

Climbing/Beam Walk Test

The beam walking assay is employed to evaluate fine motor coordination and balance in rodents. The objective of this test is for the rodent to maintain an upright position while walking along a narrow elevated beam to reach a secure platform. The assessment spans three consecutive days: two days of training followed by one day of testing. Performance on the beam is assessed by

recording the time taken for the rat to traverse the beam and the number of paw slips that occur during the task.

The methodology and procedure utilized in this study are adapted from the protocols outlined by Southwell [7] and Carter et al. [8]. The beam apparatus comprises 1-meter beams with a flat surface width of either 12 mm or 6 mm, positioned 50 cm above the tabletop on two supports. At the end of the beam, a black box serves as the endpoint. To entice the rat to the finish point, nesting material from the home cages is placed inside the black box. An aversive stimulus is created by a lamp (equipped with a 60-watt light bulb) positioned above the starting point. The time taken to cross the central 80 cm section is recorded using two motion detectors: one at 0 cm to initiate the timer and another at 80 cm to stop it. A nylon hammock (obtainable from a local fabric store) is suspended below the beam, approximately 7.5 cm above the tabletop, to cushion any potential falls.

Handgrip Test

The force transducer is connected to a bar or grid, allowing the measurement of the force exerted when pulling on the bar at regular intervals (e.g., weekly) throughout a specific experimental timeframe. Due to its simplicity and cost-effectiveness, the grip bar strength dynamometer is widely utilized in vivo to monitor diminished limb strength (both forelimbs and hind limbs) resulting from disease progression or prolonged physical activity in mdx mice. It is also employed to assess the effectiveness of therapeutic interventions (such as drugs, genetic modifications, or cell-based therapies) in reducing muscle weakness in dystrophy. The novel method of evaluating forelimb grip strength was adjusted from the traditional approach by reorienting the system vertically. This modification was expected to enhance the mice's motivation to maintain their grip on the equipment's bar.

Navigation Test Procedures

Principle: The navigation box is made of various chambers interconnected and interwoven to a final exit compartment. A trained rat with normal neurologic condition will navigate to the last compartment within 5 minutes, those with memory impairment would not.

Procedure:

A rat each was introduced into the navigating box test. Time taken (in 5 minutes) for each mouse to navigate from the origin to the last (exit) compartment was noted.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by the Newman-Keuls test for multiple comparisons. A confidence level of 95% and a significance level of $P < 0.05$ were considered.

Result

Table 1. Endurance test using the hand grip following exposure of test groups to various doses of the various sampled energy drinks

Groups	WEEK1	WEEK2	WEEK3
GROUP 1 CONTROL	26.600±8.0535	42.40±10.2352	57.24±2.2309
GROUP 2 Drink A (3.75mg/kg)	36.91±10.9372	74.26±20.7399	63.07*±16.441
Group 3 Drink A (7.5mg/kg)	52.20±4.08	58.00±3.47851	79.22±4.4564
GROUP 4 Drink B (3.75 mg/kg)	76.60±408	88.20±5.31635	48.26±6.1650
Group 5 Drink B (7.5mg/kg)	66.00±13.42	76.88±2.75316	52.90±4.6280

Group 6 Flunitrazepam (0.003mg/Kg)	5.600±1.03	7.400±2.61916	9.17±36.062
Group 7 Drink A +Flunitrazepam (0.003mg/kg)	45.28±20.21	29.60±7.34575	27.22±30.795
Group 8 Drink B +Flunitrazepam (0.003mg/kg)	20.60±6.38	27.40±4.82286	27.40±4.8229

Values are presented in mean ± sem, n= 5. * means values are statistically significant ($p \leq 0.05$) when compared to the control.

The study revealed the endurance pattern from the handgrip test following exposure of the test group to varying doses of the sampled energy drink and Flunitrazepam. The results showed a significant increase in Group 2 across weeks 1, 2, and 3. Group 3 showed a significant increase in task performance throughout all weeks. Group 4, which received 3.75mg/kg of energy drink B, demonstrated a significant increase in the endurance pattern in weeks 1 and 3 of the experiment. Group 5, which received 7.5mg/kg of energy drink B, showed a significant increase in time spent on task performance. Group 6, which received 0.003 mg/kg of Flunitrazepam, exhibited a significant decrease in task performance across all weeks. Group 7, which received a combination of energy drink A and 0.003mg/kg of Flunitrazepam, showed a significant upsurge in task performance in week 1 and a significant decrease in weeks 2 and 3. The combination of 0.003mg/kg of Flunitrazepam and 3.75ml/kg of energy drink B led to a significant decrease in task performance across weeks.

Table 2. Performance time in beam walk test following exposure of test groups to various sample of energy drinks of (A) and (B) in combination with Flunitrazepam

Groups	WEEK1	WEEK2	WEEK3
Group1	44.63±7.8955	46.62±5.5721	64.09±14.39

Group2	47.20±4.09	87.22±28.648	75.85±11.78
Group3	48.24±6.15	55.42±6.25	79.62±6.98
Group4	83.09±15.12	54.99±3.2789	108.26±35.81
Group5	84.10±14.65	48.05±8.3019	56.88±2.16
Group6	72.06±48.0310	92.02±5.53	136.16±59.85
Group7	78.07±22.43	89.07±20.237	77.138±26.6321
Group8	121.20±43.23	108.23±22.482	181.864±24.6593

*Values are presented in mean ± sem, n= 5. * means values are statistically significant ($p \leq 0.05$) when compared to the control*

The study also revealed the time spent on task performance in the beam walk task following exposure of the task group to various doses of the sampled energy drinks. Groups 2, 3, and 5 showed a significant increase in task performance characterized by an increase in time spent traversing the beam. However, the group that received energy drinks A and B in combination with Flunitrazepam showed a significant deterioration in task performance. During week 2 of the experiment, groups 5, 6, 7, and 8 showed a significant increase in time spent on task performance, while in week 3, there was a significant decrease in task performance across all groups compared to the control group. The significant increase in task performance in the beam walk test across the test groups exposed to various doses of energy drinks indicates dysfunction in motor coordination and balance.

Table 3. Performance time in navigational task test following exposure of test groups to various sample of energy drinks of (A) and (B) in combination with Flunitrazepam

Groups	WEEK1	WEEK2	WEEK3
Group1	10.60±10.6000	27.65±9.0169	25.60±5.8617
Group2	06.00±12.2025	30.032±10.803	110.404±8.539
Group3	5.60±8.38809	29.20±2.05913	121.20±2.8178
Group4	5.800±8.5639	28.80±8.87356	130.29±12.048
Group5	4.26±11.3083	49.85±5.08998	137.206.94550
Group6	38.83±8.82377	84.06±52.3377*	124.69±11.564
Group7	38.83±8.82377	120.23±32.912	130.63±11.035
Group8	27.83±23.2407*	108.22±35.0024*	196.24±24.0068*

*Values are presented in mean ± sem, n= 5. * means values are statistically significant (p≤0.05) when compared to the control*

The results of the navigational maze test also revealed the effects of these energy drinks on cognito-motor functions in Wistar rats exposed to these energy drinks and in combination with Flunitrazepam. There was a significant increase in time spent on task performance across the test groups. This shows that cognito-motor activities were greatly affected by the sampled test with longer consumption duration. The results from the navigational maze test revealed a significant alteration in the pattern of spatial awareness and orientation in Wistar rats. This indicates a

downregulation of sensory perception and integration, including spatial reasoning, in rats exposed to the substances. The results also showed that visual motor coordination and selective attention were highly influenced by the action of the energy drinks, which is common in neurodevelopmental disorders.

Discussion of findings:

Energy drinks are a popular choice for individuals looking for a quick boost of energy and alertness. Some of the most popular energy drinks available in the Nigerian market include: Monster energy drink, fearless energy drink, red bull, predator, etc. These beverages typically contain high levels of caffeine and other stimulating ingredients that can provide a temporary increase in mental and physical performance [9]. However, the addition of certain substances, such as flunitrazepam, can have potentially harmful effects on the body and brain. Flunitrazepam, commonly known as Roofies or the date rape drug, is a strong sedative that is often used to treat insomnia and anxiety disorders. When combined with energy drinks, the stimulant effects of the caffeine can mask the sedative properties of the flunitrazepam, leading to an increased risk of overdose and negative side effects. The present study has provided insightful findings on the effects of combining energy drinks with flunitrazepam, particularly focusing on their impact on endurance, motor coordination, and cognitive functions in Wistar rats. Our research has shown that energy drinks alone can significantly enhance endurance and task performance, which is consistent with the known effects of caffeine, the primary stimulant in these drinks. Caffeine's role in boosting alertness and performance is well-documented, making energy drinks a popular choice for those needing to sustain prolonged mental and physical exertion [10]. However, when we introduced flunitrazepam, a potent benzodiazepine commonly

used as a sedative and muscle relaxant, into the mix, the results were notably different. Flunitrazepam acts on the central nervous system by enhancing the neurotransmitter GABA, leading to pronounced sedative effects [11]. In our study, the combination of flunitrazepam with energy drinks led to a complex interaction that significantly affected the endurance performance cognitive and motor behavior. Initially, during the first week of administration, we observed that the combination of energy drinks and flunitrazepam prolonged the endurance pattern in Wistar rats. This could be attributed to the initial stimulatory effects of caffeine masking the sedative effects of flunitrazepam [12]. However, as the study progressed, this combination resulted in a significant decline in both cognitive and motor functions. The rats demonstrated decreased exploratory behavior and increased errors in navigational tasks, indicating impaired cognitive function and motor coordination. These findings are particularly important as they highlight the potential risks of mixing stimulants like caffeine with sedatives such as flunitrazepam. The initial masking of sedative effects by caffeine can lead individuals to underestimate the degree of impairment, increasing the risk of overdose and adverse effects in humans. This is a critical consideration for public health, especially given the popularity of energy drinks and the potential misuse of prescription sedatives. Our study aligns with previous research, such as the studies conducted Smith et al. [13, 14], which also observed that the combination of energy drinks and flunitrazepam can lead to short-term increases in endurance and physical performance but causes significant cognitive impairments as dosage and duration of consumption increase. The active ingredient in flunitrazepam works by enhancing the effects of the neurotransmitter gamma-aminobutyric acid (GABA) in the brain, which results in a calming and sedative effect [11]. However, it is also known for its potential for abuse and misuse, particularly in cases of drug-facilitated sexual assault. The sedative and amnesic effects of flunitrazepam has been reported to

impair cognitive function and memory, making individuals vulnerable to exploitation and manipulation[12]. There has also been reports of flunitrazepam-induced immobilization, decreased exploratory behavior, and overall sedation [13], these reported effects and alongside interactions of the active components together with energy drinks could have been responsible for the sharp cognitive and motor decline as seen in the exploratory tasks and endurance patterns. A study conducted by [9]also found that the combination of energy drinks and flunitrazepam can lead to a short-term increase in endurance and physical performance. Participants who consumed the mixture were able to run for longer periods of time and perceived their exertion levels to be lower than when consuming energy drinks alone [17]. However, as the dosage and duration of consumption increased, participants also reported feeling more fatigued and experiencing cognitive impairments, such as difficulty concentrating and memory problems [14].In another study [15], it was found that high doses of flunitrazepam in combination with energy drinks can lead to significant changes in cognitive function, endurance patterns and motor function decline. Participants who consumed the mixture were more likely to make errors on cognitive tasks and had slower reaction times compared to those who consumed energy drinks alone or a placebo [16]. These findings suggest that the interaction between flunitrazepam and caffeine can have detrimental effects on cognitive performance. A similar study [19] on energy drinks and alcohol consumption also found that the combination of these two substances can lead to severe damage in heart and muscle tissues. They further explained that this damage can affect gas exchange, result in hypertriglyceridemia, and impact glycemia. The work demonstrated that the combination of energy drinks and alcohol resulted in more damaged cells and abnormal tissue structures compared to when these substances were consumed individually.

Given these findings, it is imperative to raise awareness about the dangers of combining energy drinks with sedatives like flunitrazepam. There is a need for regulatory measures to control the availability and use of these substances, especially among vulnerable populations [18]. Furthermore, our study underscores the necessity for further research to explore the long-term effects of such combinations on health and to develop guidelines for safer consumption practices.

Conclusion

While the combination of energy drinks and flunitrazepam may offer short-term benefits in terms of endurance, the significant decline in cognitive and motor functions cannot be overlooked. These substances interact in ways that could pose serious risks to individuals, particularly when used without proper medical supervision.

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