Effects of Energy Drinks and Flunitrazepam on Some Cardiac Biomarkers, and Oxidative Stress in Wistar Rats.

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ABSTRACT

This study was conducted to investigate the effects of some energy drinks on cardiac biomarkers (tropin and creatinine phosphokinase) and oxidative stress in male Wistar rats. 45 Wistar rats were divided into 8 groups. Group 1 received distilled water; Group 2 received an 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. The result showed that in Group 2, there was a significant increase ($p<0.05$) in cardiac troponin kinase and no significant change in bicarbonate. Group 3 showed a significant increase ($p<0.05$) in the level of cardiac troponin, a significant increase ($p<0.05$) in creatine kinase, and no significant change in bicarbonate. Group 4 showed a significant ($p > 0.05$) decrease in cardiac troponin and creatine kinase and no significant change in bicarbonate. Group 5 showed a significant ($p > 0.05$) decrease in cardiac troponin and creatine kinase and no significant change in bicarbonate when compared to the control group. Group 6 showed a significant ($p<0.05$) increase in cardiac troponin and a decrease ($p>0.05$) in creatine kinase, but no significant change in bicarbonate. Group 7 showed a significant decrease in cardiac troponin and creatine kinase and no significant change in bicarbonate level when compared to the control group. Group 8 showed a decrease in cardiac troponin and creatine kinase. It was concluded therefore that the consumption of energy drinks and Flunitrazepam led to an increase in oxidative stress and cellular damage.

Keywords: Flunitrazepam, Energy drinks, Cardiac Biomarkers, Oxidative stress, creatine kinase.

INTRODUCTION

Energy drinks have become increasingly popular due to their stimulating effects, often used to combat fatigue and enhance physical and mental performance [1]. These beverages typically contain high levels of caffeine, taurine, and other stimulating compounds [2]. However, concerns have been raised regarding their potential adverse effects on cardiovascular health, particularly when consumed in combination with other substances such as drugs or alcohol. Flunitrazepam, a potent sedative-hypnotic drug, is known for its central nervous system depressant effects and is sometimes misused in combination with energy drinks [3].

Cardiac biomarkers, including troponin and creatinine phosphokinase (CPK), are critical indicators of cardiac muscle injury and can provide valuable insight into the potential cardiac effects of energy drinks and other substances [4]. Additionally, oxidative stress, characterized by an imbalance between free radicals and the body's antioxidant defenses [5], has been implicated in various pathological conditions, including

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cardiovascular diseases [6].

Several studies have reported adverse cardiovascular effects associated with energy drink consumption. For instance, a study by Alsunni et al., [7] found that energy drink consumption was associated with a significant increase in heart rate and blood pressure in healthy adults. Similarly, a study by Shah and colleagues [8] reported that consuming energy drinks was linked to a higher likelihood of experiencing negative cardiovascular events, such as heart attacks and sudden cardiac death.

Despite the widespread consumption of energy drinks and the potential risks associated with their use [9], limited research has investigated the combined effects of energy drinks and flunitrazepam on cardiac biomarkers and oxidative stress. Therefore, this study aimed to evaluate the impact of selected energy drinks and flunitrazepam on troponin, creatinine phosphokinase (CPK), and oxidative stress in Wistar rats.

By elucidating the effects of these substances on cardiac biomarkers and oxidative stress, this research seeks to contribute to a better understanding of the potential cardiovascular implications of energy drink consumption, particularly in combination with other substances. Furthermore, the findings may have implications for public health and regulatory policies aimed at mitigating potential risks associated with the use of energy drinks and other stimulant-containing products.

MATERIALS AND METHODS

Animals

This study utilized a cohort of 45 rats, each with an average body weight of 125 grams. The animals procured from the animal facility of the Faculty of Basic Medical Science, University of Port Harcourt, were kept in an environment with a standard room temperature. The animals were provided with regular finisher feeds (Top feed, Nigeria) and water for 14 days to allow them to adapt before the commencement of the experimental procedures.

S/N	GROUP	Name of substance administered
1	Group (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)

Administration of various doses of test substances was carried out through the oral route and lasted for 28 days. The procedure for the assay of troponin and creatinine phosphokinase typically involves the following steps:

Assay of troponin and Creatinine phosphokinase

the animals were placed under mild anesthesia, and blood samples were collected from the jugular vein of the Wistar rat. To prevent clotting, the blood samples were transferred to two heparin bottles. Afterward, it was centrifuged to separate the plasma from the cellular components of the blood. An immunoassay (ELISA) was used to measure the concentration of troponin in the plasma sample and the results were

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interpreted based on the reference range for troponin level

Assay of Creatinine Phosphokinase

The blood specimens were collected from the Wistar rat via jugular vein following a mid-anesthesia and centrifuged to separate the plasma from the cellular components of the blood. the activity of creatinine phosphokinase in the plasma sample was measured using a spectrophotometric assay and Interpretation of the results was followed according to the laboratory's established reference range for CPK activity.

Malondialdehyde (MDA) Method

Fatty acid membranes undergo peroxidation in an acidic condition, MDA combines with the chromogenic reagent, 2- thiobarbituric acid, resulting in the formation of a pink-colored complex. This complex is then measured at a wavelength of 532nm to determine its concentration, which is expressed in units of micromoles per milliliter (nmol/ml). Method: Combine 0.4 ml of the remaining liquid after centrifugation with 1.6 ml of a solution containing Tris-KCl buffer, and then introduce 0.5 ml of a 30% TCA solution. Next, 0.5 milliliters of a solution containing 0.75% TBA (tert-butyl alcohol) was added to the mixture and subjected to boiling water for 1 hour. We subjected the mixture to ice cooling and centrifuged it at a speed of 4000 revolutions per minute. The transparent liquid was gathered, and the degree of light absorption was determined at a wavelength of 532nm, with distilled water serving as a reference [10].

Superoxide Dismutase Method

This dismutase, SOD, can stop adrenaline from spontaneously oxidizing at a pH of 10.2. This serves as the basis for a simple test to measure its activity. Method: We mixed a 0.2 ml portion of the sample with distilled water to create a 1:10 dilution. Next, we combined 200 ul of the diluted sample with 2.5 ml of a carbonate buffer solution with a concentration of 0.05 mM and a pH of 10.2. We commenced the reaction by introducing 0.3 ml of recently concocted 0.3mm epinephrine into the mixture, which was promptly mixed by inversion. We recorded an increase in absorbance at 480nm for 30 seconds to 2.5mins. Unit: u/ml [11]

Statistical Analysis

Statistical analysis of results was done using the standard package for social science (SPSS version 20.0). The results were analyzed using the one-way analysis of variance (ANOVA), with a significant difference at $p<0.05$. LSD and turkeys' multiple comparisons were used to test for significant differences between the groups. The results are presented as the mean ± standard error of the mean.

RESULTS

Table 3.1 reveals the result of heat enzymes and Bicarbonate following the administration of doses of energy drink and flunitrazepam. The result showed that Group 2 (drink A, 3.5ml/kg) was statistically significant with increment of HCO_3 (mEg/L), while there was a significant difference observed in group 4 (drink B, 3.5ml/kg) on troponin and CK-MB. Group 6 (Flunitrazepam, 0.003mg/Kg) also showed statistical differences with troponin and CK-MB

Table 3.2 further revealed the result of oxidative stress parameter following the administration of various doses of the samples energy drinks and Flunitrazepam The result showed that Group 2 which received 3.75mg/kg of energy drinks (A) had a significant decrease in SOD and an increase In MDA. Group A which received the doses of energy drink showed a significant increase in MDA and reduce SOD furthermore the

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result showed that energy drinks in combination with Flunitrazepam cause more increase in MDA.

Table 3.1 Result of Heart Enzymes and Bicarbonate following administration of doses of Energy Drink A, B, and/or Flunitrazepam

GROUPS	TROPONIN.I (ng/L)	CK-MB (IU/L)	HCO3 (mEq/L)
Group 1 Control	45.60±2.003	22.60±0.82	23.20±0.12
Group 2 Drink A (3.75ml/Kg)	43.40±1.02	28.60±0.71	28.20*±0.31
Group 3 Drink A (7.5ml/Kg)	49.20±0.08	25.20±1.20	22.60±0.23
Group 4 Drink B (3.75 ml/Kg)	38.60*±0.20	19.40*±0.97	26.40±0.71
Group5 Drink B (7.5ml/Kg)	40.40±0.12	19.80*±0.01	24.80±0.01
Group6 Flunitrazepam (0.003mg/Kg)	58.60*±0.31	30.60*±1.00	25.60±0.23
Group7 DrinkA + Flunitrazepam (0.003mg/Kg)	43.40±0.21	19.40*±0.03	24.80±0.21
Group8 Drink B+ Flunitrazepam (0.003mg/Kg)	17.60*±0.20	8.60*±0.31	26.80±0.12

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

Table 3.2 Result of Oxidative Stress Parameters

Groups	SOD (U/ml)	MDA (muMol/L)
Group 1 Control	**0.21±0.01**	**0.41±2.43**
Group 2 Drink A (3.75mg/kg)	**0.17±0.01**	**0.54±8.22**
Group 3 Drink A (7.5mg/kg)	**0.15*±0.01**	**0.52±0.02**
Group 4 Drink B (3.75 mg/kg)	**0.15*±0.01**	**0.51±0.11**
Group 5. Drink B (7.5mk/kg)	**0.16*±0.01**	**0.90±0. 012**
Group 6. flunitrazepam (0.003mg/kg)	**0.16±0.02**	**1.02±0.43**
Group 7.Drink A+ flunitrazepam (0.003mg/Kg)	**0.18±0.01**	**1.23±0.26**
Group 8. Drink B+ Flunitrazepam (0.003mg/kg)	**0.16±0.01**	**1.8±0.34**

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

DISCUSSION

Table 3.1, Reveals the results of heart enzymes and bicarbonate Group 2 Which was administered with (3.75 mg/kg) of energy drink (A)showed a significant decrease in cardiac Troponin when compared to the control group, for this group Creatine kinase showed significant increase when compared to control the group.

Group 3 Which was administered with 7.5 mg/kg of energy drinks, (A) also showed a significant increase in the level of cardiac troponin, a significant increase in creatine kinase, and no significant changes in bicarbonate. Group 4 which received (3.75 mg/kg) of energy drinks (B) showed a significant decrease in cardiac troponin, a significant decrease in creatine phosphokinase, and a significant increase in bicarbonate. Group 5 (7.5mg/kg) of energy drink (B) showed a significant decrease in cardiac Troponin, a significant decrease in Creatine kinase, and no significant changes in bicarbonate. Group 6 which received (0.03 ml/k of flunitrazepam showed a significant increase in cardiac Troponin and a significant decrease in creatinine kinase and also a significant increase in bicarbonate. Group 7 which received (0.03 mg/kg) flunitrazepam

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and (3.75 mg/kg) of energy drink (A) showed a decrease in cardiac Troponin which was not statically significant, but a significant decrease in creatine kinase and no significant change in bicarbonate.

Group 8 which received (3.75 mg/kg) and (0.03 mg/kg) showed down surge of cardiac troponin and creatine kinase and a significant increase in bicarbonate.

These findings suggest that the different doses of energy drinks and the combination with Flunitrazepam have varying effects on cardiac enzyme levels and bicarbonate. These results highlight the potential impact of these substances on cardiac function and metabolism.

The changes in cardiac troponin levels observed in the result may have significant implications for cardiac health and function. Cardiac troponin is a protein that is released into the bloodstream when there is damage to the heart muscle, such as during a heart attack or other cardiac injury [12]. An increase in cardiac troponin levels is often indicative of myocardial damage or stress on the heart [13], Elevated troponin levels can be seen in conditions such as myocardial infarction, myocarditis, heart failure, and other cardiac conditions [14].

As evident in the result, the significant increases and decreases in cardiac troponin levels in response to different doses of energy drinks and the combination with Flunitrazepam suggest potential effects on cardiac muscle damage or stress. These changes could indicate potential cardiac injury or strain, which may have implications for overall heart health and function. However further investigation and clinical studies would be necessary to fully understand the implications of these changes in cardiac troponin levels and their potential impact on cardiovascular health. However, the observed changes in cardiac troponin levels raise concerns about the potential effects of energy drinks and the combination with Flunitrazepam on cardiac health and the need for further research in this area.

Oxidative stress is a condition that occurs when the body's antioxidant defense mechanisms are overwhelmed by the production of reactive oxygen species (ROS) and other free radicals [15]. These ROS and free radicals can cause damage to cellular components such as lipids, proteins, and DNA [16]. To assess the impact of energy drinks and Flunitrazepam on oxidative stress parameters was measured following the administration of various doses of these substances. The results of the study revealed that Group 2, which received a dose of 3.75mg/kg of energy drinks (A), experienced a significant decrease in Superoxide dismutase (SOD) and an increase in Malondialdehyde (MDA). SOD is an antioxidant enzyme that plays a crucial role in protecting cells from oxidative stress by converting superoxide radicals into less harmful molecules [17]. On the other hand, MDA is a marker of lipid peroxidation and is an indicator of oxidative damage to cell membranes [18]. Furthermore, the results showed that Group A, which received the doses of energy drink, exhibited a significant increase in MDA and a reduction in SOD. This suggests that the consumption of energy drinks may lead to an increase in oxidative stress and cellular damage. Moreover, the study also revealed that the combination of energy drinks and Flunitrazepam caused even more of an increase in MDA. Flunitrazepam is a benzodiazepine drug that is commonly used as a sedative and hypnotic [19]. The combination of energy drinks and Flunitrazepam may have a synergistic effect on oxidative stress parameters, leading to even greater cellular damage. Overall, the results of this study suggest that the consumption of energy drinks and Flunitrazepam may lead to an increase in oxidative stress and cellular damage, which could have negative health consequences [20]. It is important to be aware of the potential risks associated with the use of these substances and to consume them in moderation.

CONCLUSION

Based on the results presented, it is evident that the different doses of energy drinks and the combination with Flunitrazepam have diverse effects on cardiac enzyme levels and oxidative stress parameters. The findings highlight the potential impact of these substances on cardiac function, metabolism, and oxidative

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stress. Specifically, the changes in cardiac troponin levels observed in the study suggest potential effects on cardiac muscle damage/stress, which may have implications for overall heart health and function. Moreover, the alterations in Superoxide dismutase (SOD) and Malondialdehyde (MDA) levels indicate an increase in oxidative stress and cellular damage associated with the consumption of energy drinks and the combination with Flunitrazepam. These results raise concerns about the potential negative health consequences of consuming energy drinks and Flunitrazepam, emphasizing the need for further research in this area. It is crucial to be aware of the potential risks associated with the use of these substances and to consume them in moderation. Further investigation and clinical studies are necessary to fully understand the implications of these findings and their potential impact on cardiovascular health and oxidative stress.

Ethical consideration

The study was approved by the ethics and research committee of the University of Port Harcourt, Nigeria

Competing interest

None

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