

Original Research Article

Hepatic & Renal Toxicity of Energy Drinks, A serious Health Risk, animal model study

Comment [CN1]: Please change your title. Make it more interesting

Number of Pages: 13

Number of Figures: 6

Number of Tables: 2

Abstract

The recent years have seen a significant increase in the individual consumption of energy drinks across the globe. As a result, notable concerns continue to rise both among the public and among the scientific community, concerning the health effects that energy drinks pose to individuals. This study therefore examines the adverse effects of energy drinks on health, using normal albino rats as specimens. The specimen ~~were~~ ~~was~~ then divided into two groups consisting of five rats each, with one group given energy drinks while the control group given normal drinking days for a period of 21 days. The specimen ~~were~~ ~~was~~ then sacrificed and their blood samples used for biochemical and histological studies. The study findings reveal that that energy drink consumption significantly increased the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), ~~U~~urea and ~~C~~reatinine levels when compared with the control group. In addition, the study demonstrates that energy drink consumption significantly decreased body weight and ~~O~~rgan to body weight ratios when compared with the control group. The study

Comment [CN2]: How many animal??

further recognizes that the consumption of energy drink is associated with significant alterations in serum toxicity markers, bodyweight, organ body weight and histopathological changes. The study therefore recommends that need for public health education to educate individuals; especially youths about the risks of consuming highly caffeinated energy drinks. The study also recommends the need for further studies in order to minimize the harm excess and long-term consumption of energy drinks presented to the public.

Keywords: Energy Drinks, ~~Caffeinated drinks~~, Weight loss drinks, non-alcoholic energy drinks.

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Introduction

The past decade has seen over 500 different types of energy drinks in the world markets [1]. It is beyond doubt that the energy drinks sector is significantly booming, with its sales increasing to over \$12.5 billion in 2012 alone, a 60% increase from 2008 [2]. Compared to other soft drinks, energy drinks are relatively new to the market, with Japan launching the first energy drink in 1960. The first energy drink first arrived in Europe in 1987, then spreading across Europe and eventually appearing in the United States in 1997. In spite of its increasing popularity, energy drinks seem to lack a standard definition, as it is commonly described as a non-alcoholic drink that consists of caffeine, vitamins, taurine among other combinations. In most cases, energy drinks are widely marketed for its actual benefits for improving individual performance and increasing energy [2]. Despite the fact that energy drinks is quite a new phenomenon compared to other beverages, recent trends suggest otherwise, as it continue to gain popularity among the younger population, who often mix it with alcohol [3]. For instance,

according to Food and Beverage Committee estimates 5 million young men consume more than 5 million energy drink cans daily, worth SR15 million [4]. Elsewhere, the European Food Safety Authority (EFSA) set up a study that aimed to collect data on the consumption of EDs in 16 different countries within the European Union in 2011. The study revealed that 68% of adolescents, 30% of adults and 19% of children consumed EDs [2].

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Irrespective of their lack of alcoholic content, EDs are highly caffeinated that pose numerous adverse effects to consumers [5, 6]. For example, one single can of Red Bull, 250 mL (one of the most consumed brand of EDs) contains 80 mg of caffeine (or 0.32 mg/mL) and 1000 mg of taurine (or 4mg/mL) as major components. In many acute clinical human trials, some over-the-counter energy drinks have shown a positive stimulation of resting energy expenditure (REE) [1, 4]. With an increase in energy expenditure at rest, more energy is being expended as long as the chemicals are within the system. A greater amount of calories being expended at rest translates into weight loss over a period. Furthermore, several research studies have demonstrated that short-term thermogenic drink (TD) ingestion does not result in adverse health effects, making it safe for healthy individuals [7, 8]. Globally, governments are banning the sale of energy drinks especially at government offices, educational and health facilities as well as an advertisement for energy drinks on social, cultural and sports events [7]. Studies on the safety of energy drinks have been inconsistent. It has been reported that long-term exposure to the various components of energy drinks may result in significant alterations in the cardiovascular system [10, 11]. However, research is yet to be conclusive on the safety or otherwise of energy drink consumption [12, 13]. Several government officials are expressing concerns about the safety and efficacy of the use of energy drinks [14]. The present study is therefore aimed at investigating the effects of energy drink in normal albino rats.

Materials and Methods

Animals: Wistar albino rats approximately of the same age (8-10 weeks), weighing 200-220 g were used. Animals were kept at constant temperature (22 ± 3 °C), humidity (55%) and light-dark conditions (12/12 h light/dark ratio). Animals were fed on a standard animal chow diet and drinking water ad libitum.

Treatment schedule:

Ten Wistar rats weighing 200-220 g were assigned into two groups of five rats per group. Group 1 rats were given ED; group 2 rats were given normal drinking water and served as controls. The treatment lasted for 21 days, after which the animals were sacrificed and their blood and organs were collected for biochemical and histological studies.

Body weight and organ weight:

Body weights of rats of each were recorded at twice-weekly intervals, and final body weights were recorded after 24 h following last dosing for each group. Animals were sacrificed under anaesthesia and organs (liver, heart and kidney) were dissected out, cleared free of fat and connective tissues by washing in ice-cold physiological saline (0.9%) and weighed on electronic balance. The organ body weight index (OBWI) was calculated as (organ weight/body weight) × 100. Fresh isolated sera were used for serum liver and renal injury biomarkers study according to protocols by the manufacturer. Then serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated as per standards [8].

Histological evaluation:

Tissues will be fixed in freshly prepared 10% formalin solution and processed for paraffin sections. Sections will be cut at 5 µm with a rotary microtome. Sections were stained with hematoxylin and eosin for histological evaluation.

Comment [CN5]: Name the drug that you used for anaesthesia

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Statistical analysis:

All data will be expressed as mean \pm SEM, and statistical analysis will be done using one-way ANOVA test. Significance between the control group and treated groups will be performed using Dunnett's -'t' test.

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Results

The effects of the administration of the caffeinated energy drinks on the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine are outlined in (Table 1). The results showed that energy drink consumption significantly increased the AST, ALT, urea, and creatinine levels when compared with the control group. This confirms the toxic effect of such drinks on normal laboratory values. On the other hand, the effects of the administration of the caffeinated energy drink on the Organ to body weight ratios was clearly expressed in Table 2. The study further reveals energy drink consumption significantly decreased body weight and organ to body weight ratios when compared with the control group.

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Histological sections of liver, kidney and heart tissues showed well-preserved respective tissue architecture. The liver of energy drink treated rat showed vacuolar degeneration of some hepatocytes (empty foci devoid of liver tissue) as well as focal hepatic hemorrhage, kupffer cell activation and dilatation of hepatic sinusoids (Fig. 1 and 2).

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The histopathological study of the kidneys of the rats in the test and control groups appeared normal with no irregularities or abnormalities (Fig. 3 and 4). Which may relate that such drinks histological toxicity would require a longer period of consumption.

Comment [CN15]: Can you explain the normal features of kidney tissue?

Histological examination of the heart shows no significant changes in the diameters of masts. Interstitial fibrosis and disarrangement of myocytes were slightly increased in the 21 days energy drink treatment (Fig. 5 and 6).

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Discussion

Several studies suggest that the adverse effects caused by EDs intake are due to the excess intake of caffeine. Most of these studies have demonstrated that such adverse effects were witnessed after a long period of consumption. For example, Steinke et al. conduct a prospective study to determine the effects of EDs on blood pressure and heart rate in humans. The results of this study reveal that after 4 hours of EDs consumption, individuals blood pressure and heart rate increased significantly, whereas diastolic blood pressure increased within 2 hours of ED consumption [15]. However, the current study finds no significant changes in the diameter of the rats' heart masts. Since these findings do not provide sufficient evidence EDs consumption causes heart failures, it is important to conduct further research to validate the amount of EDs that cause negative effects [16, 17]. Elsewhere, Lockwood et al. perform a randomized placebo-controlled study to determine the effect of EDs on fat mass in humans following intensive exercise. The results of the study indicate that after EDs consumption and exercise, fat mass and body mass decreased significantly compared to those individuals subjected to placebo and exercise without EDs (18). Similarly, the current study also reveals that the body and organ weight of rats on EDs significantly reduced compared to the control group. In addition, other studies have noted that the consumption of moderate amounts of EDs improved individual mental performance including concentration, choice reaction time and memory. However, the study notes that higher consumption of EDs significantly worsens individual mental

performances (19, 20). Likewise, the findings of the current research reveal the same results in rats on EDs. The study reveals rats on EDs had significantly increased the AST, ALT, Urea, and Creatinine levels when compared with the control group.

Conclusion

Based on the results of this study, it is evident that the consumption of energy drink is associated with significant alterations in serum toxicity markers, bodyweight, and organ body weight. In addition, histopathological finding also shows considerable histological changes. The study **therefore** recommends that energy drinks should be consumed with caution. Public health education is urgently needed to educate individuals; especially youths about the risks of consuming highly caffeinated energy drinks. The study also recommends the need for further studies in order to minimize the harm excess and long-term consumption of energy drinks presented to the public.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1: Effect of oral administration of the Energy drink for 21 days on the serum toxicity markers.

Comment [CN17]: Any significant finding???. If significant, no symbol added in the Table 1.

Groups	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	64.15 ± 6.62	146.2 ± 8.4	33.35 ± 3.32	0.34 ± 0.05
Energy Drink	72.7 ± 8.7	155.50 ± 7.50	37.55 ± 4.5	0.45 ± 0.04

Table 2: Effect of oral administration of the Energy drink for 21 days on **organ to body weight ratios**.

Treatment	Body weight	Organ to Body Weight Ratios (g/100g)		
		Heart	Liver	Kidney
Control	224.5±8.25	0.36±0.05	3.60±0.70	0.58±0.02
Energy Drink	215.8±5.25	0.39±0.08	3.80±0.85	0.50±0.05

Comment [CN18]: Body weight here, is it cumulative after 3 weeks??? What about weekly body weight? Did you measure/monitor it?

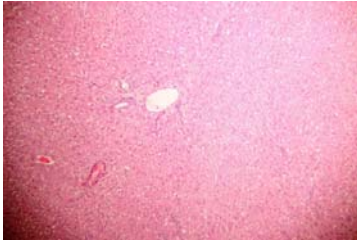


Fig:1 Control liver 100X

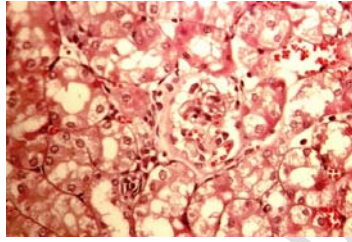


Fig:2 Control Kidney 400x

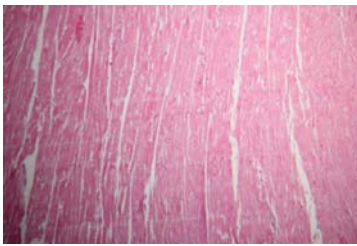


Fig: 3 Control Heart 100x

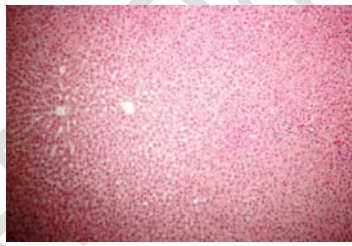


Fig:4 ED-treated liver 100X

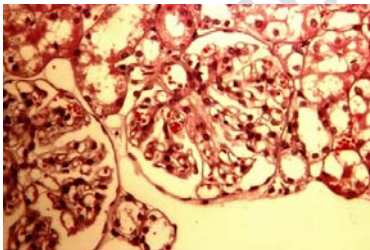


Fig:5 ED-treated Kidney 400x

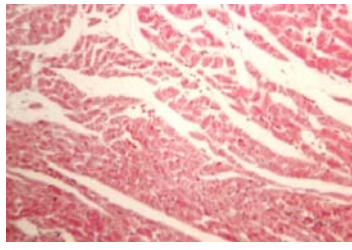


Fig: 6 ED-treated Heart 100x

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Comment [CN23]: Too small. suggest to take picture under 400X magnification

Comment [CN22]: For each figure, you should add footnote to explain the normal or abnormal findings. Do not assume all readers know the architecture of liver, kidney and heart of the animal.