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Changes in biochemical and sperm parameters of rats drinking energy drinks

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Abstract

Tiger energy drink is used in this research to see the effect on serum lipid profile, liver enzymes, and kidney function, also observing changes in sperm parameters. Sixteen male rats were divided into four groups, each consisting of four rats. The control group was given water and rat feed, while groups 1, 2, and 3 were given water with Tiger energy drink (25%, 50%, and 75%, respectively) for eight weeks. The results showed that the effect of energy drinks on lipid profile decreased cholesterol, TG, and LDL, while there was a slight increase in HDL. Treatment with wild Tiger energy drink generally caused insignificant increases in GOT and GPT in both G2 and G3 groups as compared to the control. However, in the G1 group, GPT and GOT significantly increased. Accordingly, ALP concentration was significantly increased in all experimental groups compared to the control group. Sperm quality declined as the concentration of energy drinks consumed increased. The potential adverse effects of energy drinks, at their specific dosages, have been determined to include hepatic and renal impairment, as well as alterations in serum lipids. Additional research is required to investigate the specific mechanism by which energy drinks impact spermatogenesis, either through the modulation of endocrine hormones or other metabolic pathways.

Keywords: Energy drink, Caffeine, Lipid profile, Liver function, Renal function.

1. Introduction

Energy drinks are carbonated beverages that are devoid of alcohol and are specifically formulated to provide the customer with a boost of energy. The global use of energy drinks has witnessed a significant rise since its debut in the markets in the 1960s [1]. Energy drinks are composed of several components, including caffeine, taurine, guarana, glucuronolactone, vitamins, and carbs, alongside water. Carbohydrates are sources of energy, and caffeine excites the central nervous system [2, 3].

Based on the guidelines provided by the U.S. Food and Drug Administration (FDA) 400 milligrams of caffeine per day, or four to five cups of coffee, is deemed to be a threshold below which adverse effects are not typically observed in the context of healthy adult individuals. Any quantity over the aforementioned threshold has the potential to induce major issues in adulthood, with toddlers and teenagers being particularly affected. Significantly, the quantity of caffeine included in non-prescription items is restricted to a maximum of 200 mg per dosage, whereas energy drinks are not subject to any limitations [4].

The predominant health risks associated with energy drinks seem to be associated with the consumption of caffeine, caffeine-like chemicals, and other substances found in energy drinks, such as taurine, which may potentially interact with caffeine [5].

Based on the findings of the NOMISMA-ARETÉ Consortium for the European Food Safety Authority (EFSA), it was observed that the largest incidence of eating disorders (ED) was documented among teenagers (68%), with adults (30%) and children (18%) exhibiting lower prevalence rates [6].

These beverages effectively mitigate the symptoms of fatigue that occur naturally in the body. As a result of this characteristic, a considerable number of individuals opt to consume them in order to enhance their productivity or optimize their overall performance. The demographic most commonly associated with the misuse of such beverages comprises primarily of athletes and students who seek to enhance their focus, as well as their physical and cognitive performance over extended periods of time [7]. When examining the constituents of energy drinks and acknowledging their inclusion of psychoactive substances known for their potent stimulating effects, it becomes crucial to address the matter of caffeine levels. These concentrations can range from 50 mg per 250 mL can to 505 mg per 1 L bottle, thereby posing a potential risk of poisoning or overdose [8]. In addition to caffeine poisoning, there is an established correlation between the consumption of energy drinks and the occurrence of seizures. [9], heart problems [3], and decreased sperm quality [10].

There has been a notable rise in the number of repor-

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ted instances of substantial and detrimental health issues resulting from the consumption of energy drinks in recent times. In 2013, the number of emergency interventions related to energy drinks, as reported by the US Substance Abuse and Mental Health Services Administration, experienced a twofold increase, rising from 10,068 in 2007 to surpassing 20,000 in 2011[11]. One significant limitation in comprehending the association between energy drinks and the negative consequences of their usage is in the limited knowledge regarding the toxicity of the diverse substances included inside them. Nevertheless, considering the documented instances of health issues related to energy drinks and the widely recognized physiological impacts of their active components, it is probable that the observed negative consequences of energy drinks can be attributed to their compositions [12].

The primary objective of this study is to assess the impact of energy drinks on the lipid profile, hepatic and renal functions, and sperm parameters in male albino rats. The biochemical markers present in blood serum will be examined with the sperm parameters, which encompass motility, concentration, and morphology.

2. Materials and Methods

2.1. Energy drink

The most popular and available energy drink in Iraq is Wild Tiger, and it was purchased from the local market. The ingredients are water, sugar, citric acid, trisodium citrate, caffeine, taurine, glucuronate, vitamin (B2, B6, B12), pantheonic acid, niacin, benzoic acid, colors, and flavorings.

2.2. Experimental animals

A total of sixteen male Wistar albino rats were utilized for the study. The rats were housed in conventional metallic cages, with four rats per cage. They were maintained in a temperature-controlled environment, with a mean temperature of 24 ± 2 ℃. Additionally, the rats were subjected to a 12-hour light-dark cycle, alternating between periods of light and darkness. The subjects were used to the controlled laboratory environment, provided with a standardized diet, and had unrestricted access to water. The animals were properly cared for and the experimental procedures followed the guidelines outlined for the care and use of laboratory animals [13].

2.2.1. Experimental design

The animals were categorized into four groups, with each group consisting of four rats. The rats were subjected to the following treatments:

Control: Animals in this group were given drinking water and served as control.

Group 1(G1): Animals of this group were served with drinking water 25% Wild tiger energy drink (25% tiger +75% water) for eight weeks.

Group 2(G2): Animals of this group were served with drinking water 50% Wild tiger energy drink for eight weeks.

Group 3(G3): This group's animals were served with water 75% Wild tiger energy drink for eight weeks.

2.3. Determination of biochemical tests

The COBAS INTEGRA 400 plus system, a fully automated biochemical analyzer manufactured in Germany, was employed to measure the amounts of several biochemical parameters in the serum of both control and experimental groups of rats. The concentrations of serum cholesterol, triglyceride, HDL, and LDL were determined using a commercially available kit provided by CENTRONIC GmbH. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined using commercially available kits from NS, BIOTECH Co., Egypt. The assessment of renal function in this investigation was the measurement of serum creatinine and urea levels. The diacetyl monoxime method was employed to assess blood urea levels, while the alkaline picrate method (Jaffe's Method) was used to determine serum creatinine levels. These measurements were conducted using a commercially available kit from BIOLABO, France [14]. The total serum bilirubin (TB) and fasting blood glucose were assayed using a commercial kit (BIOLABO SAS).

2.4. Evaluation of fertility

The cauda epididymis was isolated from the caput and corpus areas, its weight was measured, and it was then placed in a suspension of 2 ml phosphate buffer saline at 37 °C and pH 7.2. The collection of epididymal fluid was performed by making incisions in the cauda epididymis, allowing the fluid to be collected in phosphate-buffered saline (PBS). Subsequently, a tube was used to collect the epididymal fluid enriched with sperm. The motility, concentration, viability, and abnormalities of the spermatozoa were assessed.

2.4.1. Sperm motility

The motility of spermatozoa was assessed by employing a small quantity of sperm suspension. A total of 200 motile and non-motile spermatozoa were observed utilizing an Optica microscope. Sperm motility pertains to the progressive motion of spermatozoa, and the quantification of motile and non-motile spermatozoa is not typically performed in its natural environment [15].

2.4.2. Sperm concentration

The Neubauer hemacytometer slide was utilized to assess the concentration of sperm, following the guidelines outlined in the laboratory manual established by the World Health Organization (WHO) for the testing and processing of human semen [16].

2.4.3. Sperm viability percentage

The vitality of sperm was assessed with the application of eosin-nigrosin staining in a dehydrated solution of 3% sodium citrate, following the protocol outlined by Ajayi and Akhigbe [17] .

2.4.4. Sperm abnormalities

A total of around one hundred spermatozoa were examined using a microscope to assess potential changes in sperm morphology [15].

2.5. Statistical analysis

The data were presented as the average with the standard deviation (SD) included. To analyze the data, a one-way analysis of variance (ANOVA) was conducted using GraphPad Software Inc. To evaluate the differences between the groups, Dunnett's multiple comparisons test

was performed. Results were considered to be statistically significant if the mean value was less than 0.05.

3. Results

Table 1 presents the effect of Wild Tiger energy drink on body weight gain; the control group gained weight by (19.84%) compared to the experimental groups, Group 1 (G1) lost weight by (27.37 %) of their initial weight, and the difference was significant, while in Group 2 (G2) there was a slight increase in weight (3.28%) and the Group 3 (G3) there was a slight decrease in weight (-1.5%).

Table 2 shows the effects of energy drinks on plasma lipids and glucose levels of male albino rats. A significant decrease was observed in serum cholesterol concentration in G1 and G3, while the decrease in G2 was not significant. While all groups had significantly lower TG concentrations than the control group, the difference was highly significant in G1 and G3 while in G2 was less significant. The G2 group showed an insignificant decrease in HDL levels, while G2 and G3 groups increased when compared to the control group and a significant increase was only observed in G2. LDL levels decreased insignificantly in the groups G1, G2, and G3compared with the control group. Glucose level slightly increased insignificantly in the G1 group, while there was a highly significant increase in G2 and G3 but less significant.

The effects of the administration of energy drinks on plasma liver function are shown in Table 3. Treatment with wild Tiger generally caused insignificant increases in GOT in G2 and G3 compared to control. However, in G1, there was a significant increase. GPT was significantly increased in G1, while the increase observed in GPT in G2 and G3 was insignificant. ALP concentration was significantly increased in all experimental groups compared to the control group, and the significance was higher in G2 and G3 groups.

Table 4 shows that creatinine levels were affected and increased in the experimental groups while the increase was not significant, and different doses of energy drinks did not cause a noticeable difference within the experimental groups. As for urea levels, in the G1 group, a significant rise was observed in comparison to the control group, while there was an insignificant decrease in G2 and G3 in contrast with the control group.

Table 5 presents the effect of energy drinks on sperm parameters; sperm count was decreased in all experimental groups and was only significant in Group 3. Sperm motility was decreased in all experimental groups while the decrease was higher in G1 and G3, and only G3 was significant when compared to the control group. as for sperm viability, there was a slight decrease in groups G1 and G2, but there was a noticeable decrease, and it was highly significant when compared to the control group. Sperm abnormality increased with the increased concentration of energy drink received, in G1 was lowest among experimental groups and became higher in G2 and it was highest in G3, and the increase was highly significant in the last group.

Table 1. Effects of energy drinks on body weights.

Parameters	Means ± standard deviation			
	Control (0%)	Group 1 (25%)	Group 2 (50%)	Group 3 (75%)
Initial Body Weight (g)	300.66 ± 2.51	293.33 ± 20.79	319.33 ± 13.65	328.66 ± 11.50
Final Body Weight (g)	360.33 ± 5.68	211.66 ± 18.92 ***	330.00 ± 22.91	324.33 ± 42.02
Weight Gain (g)	59.66 ± 4.04	-81.66 ± 39.71***	10.66 ± 12.09	-4.33 ± 30.82*
Weight Gain (%)	19.84 ± 1.27	-27.37 ± 11.13***	3.28 ± 3.71	-1.5 ± 9.54*

Table 2. Effects of energy drink on plasma lipids and Glucose Levels of Male albino rats.

Parameters	Means ± standard deviation			
	Control (0%)	Group 1 (25%)	Group 2 (50%)	Group 3 (75%)
Total cholesterol (mg/dl)	70.00 ± 5.29	39.00 ± 7.81**	66.00 ± 6.00	58.66 ± 6.12*
Triglyceride (mg/dl)	82.33 ± 11.67	25.33 ± 6.59****	57.00 ± 9.59**	48.00 ± 8.52***
HDL-Cholesterol (mg/dl)	34.66 ± 5.03	24.66 ± 14.36	48.66 ± 4.18*	44.33 ± 7.37
LDL-Cholesterol (mg/dl)	11.63 ± 3.75	9.20 ± 2.10	6.80 ± 5.23	4.80 ± 4.20
Serum glucose (mg/dl)	131.60 ± 8.83	137.00 ± 53.00	264.00 ± 46.80**	227.33 ± 6.35*

Table 3. Effects of energy drink on liver function parameters of Male albino rats.

Parameters	Means ± standard deviation			
	Control (0%)	Group 1 (25%)	Group 2 (50%)	Group 3 (75%)
GOT (AST)(U/L)	115.33 ± 8.38	493.33 ± 140.64***	126.33 ± 11.67	132 ± 36.51
GPT (ALT)(U/L)	58.33 ± 3.78	257.33 ± 20.10***	61.66 ± 5.85	61.66 ± 6.35
ALP(IU/L)	169.66 ± 10.40	224.40 ± 34.44*	230.26 ± 29.77**	229.86 ± 3.28**

Table 4. Effects of energy drink on creatinine and urea of male Albino rats.

Parameters	Means ± standard deviation			
	Control (0%)	Group 1 (25%)	Group 2 (50%)	Group 3 (75%)
Creatinine (mg/dL)	0.20 ± 0.10	0.30 ± 0.00	0.33 ± 0.05	0.33 ± 0.05
Urea (mg/dL)	45.00 ± 3.00	77.66 ± 14.22 *	35.66 ± 9.23	33.66 ± 12.5

Table 5. Effects of energy drinks on sperm parameters.

Parameters	Means ± standard deviation			
	Control (0%)	Group 1 (25%)	Group 2 (50%)	Group 3 (75%)
Sperm count (*5*10^4)	71.71±5.64	40±29.16	46±46.35	29.36±16*
Sperm motility	89.25±3.68	71.25±6.13	85.25±7.32	65.5±17.44*
Sperm viability	91.25±5.25	84.25±3.3	82.25±8.8	63.25±8.5***
Sperm abnormality	12±0.81	15.5±3.31	17.5±8.81	36.5±8.5***

4. Discussion

The current investigation has demonstrated that the consumption of energy drinks can result in diverse impacts on lipid profile, renal functions, liver enzymes, and sperm parameters.

Regarding the effect of consuming energy drinks on weight gain, we noticed that most gained weight was in the group that received 50% energy drink (3.2%) which is lower compared to the control group. As opposed, the other two groups even lost weight, which might be because they consumed energy drinks more than the rat chow available ad libitum, and the energy drink does not contain proteins essential for growing muscles and gaining weight. Although the study on the effect of energy drinks on decreased body weight was not encountered, most studies disagree with our results which they find that consumption of energy drinks leads to an increase in body weight at different rates compared to the control group [18, 19].

Weight loss evidence can be easily seen through the decrease in the concentration of all plasma lipids except HDL, which was slightly higher than in the control group. The only way we can explain this is because of the reduced amount of chow they consumed, considering they get their energy source from energy drinks; for most days, G3 consumed nearly a whole bottle, which led to decreased weight. These results contradict previous research where plasma lipids increased [20]. Another reason for these alterations might be caffeine, the common ingredient in all energy drinks, as reported in some studies [21, 22].

As anticipated, the administration of the energy drink resulted in a substantial elevation in glycemia, as indicated in Table 2. The 250 ml container of Wild Tiger contains 27 g of carbs and other substances, such as niacin, which have the potential to influence glucose metabolism. According to [23] and [24], The potential association between niacin supplementation in high carbohydrate diets and the diabetes epidemic has been suggested. In addition, it has been observed that niacin has the potential to elicit insulin resistance [25].

The findings of this study align with previous research conducted by Mansy et al. (2017) and Khayyat et al. (2015) [26, 27], which examined the impact of energy drink use on plasma liver function outcomes, which led to an increase in liver enzymes, especially ALP enzyme. Elevations in the blood concentrations of hepatic enzymes are considered dependable markers for the presence of liver injury caused by toxic substances. Elevated levels of serum AST, ALT, and ALP have been noticed in rats following exposure to energy beverages [28].

Kidney function tests showed increased creatinine in the groups that consumed the energy drink compared to the control, while urea only increased in group 1. These results are not an exact match to the findings of previous research. According to research, The administration of energy drinks to rats results in a considerable increase in blood urea and creatinine concentrations. Elevations in blood concentrations of urea and creatinine are commonly correlated with compromised renal function [29]. The researchers proposed that the activation of A2A adenosine receptors is inhibited by coffee, leading to higher levels of urea, uric acid, and creatinine. This, in turn, contributes to the occurrence of interstitial inflammation, heightened proteinuria, and adverse alterations in renal function and structure [30].

Energy drink consumption affected sperm parameters (count, motility, viability, and abnormality) in experimental groups compared to the control group. However, the decline in the quality of sperm was bound to increase in the concentration of energy drinks consumed as it was highly significant in G3, which consumed the highest concentration; this result is in accordance with the results of [31], who reported that a change in sperm morphology. Abnormal sperm morphology, number, and viability may reflect abnormality in spermatogenesis affected by the content of energy drinks [32]. Male fertility is contingent upon the presence of normal linear progressive sperm motility and normal sperm morphology. Additionally, several factors like as diet, lifestyle, stress, and socioeconomic status might impact the quality of semen [33].

5. Conclusion

The potential adverse effects of energy drinks on hepatic, renal, and serum lipid profiles have been excluded. Additional research is required to investigate the specific mechanism by which energy drinks impact spermatogenesis, either through the modulation of endocrine hormones or other metabolic pathways. This necessitates conducting studies on a larger sample size. When energy drinks are drunk in high quantities over an extended period, they have been observed to have a detrimental effect on sperm concentration in rats. However, other factors such as sperm motility, morphology, water intake, and symptoms of toxicity do not appear to be affected.

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Conflict of interests
None declared.

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All authors read and approved the final manuscript for publication.

Ethics approval and consent to participate
Not applicable.

Informed consent
Not applicable.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Sarwar N. Jafar did all the work alone.

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