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Effect of energy drink on blood, liver and kidney functions in male albino rats

Yaqeen Dheyaa Jabbar and Zaid Makki Mohammed AL-Hakkak*
Dep. of Ecology and Pollution, Faculty of Science, University of Kufa, Najaf, Iraq.

***Correspondence: :**
zaid.alhakkak@uokufa.edu.iq
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ABSTRACT

The objective of this study was to evaluate the effect of energy drinks on blood parameters, and liver and kidney function markers in rats. Twelve male rats were used the animals were divided into four groups each group consisting of three animals. The rats experiment were exposed to three levels of tiger energy drink exposure: mild, moderate and high tiger energy drink (0.3, 1.2 and 4.3 ml/100 g body weight/day) for 30 days. The study found that there was a significant (P≥0.05) elevation in the total leukocyte count of rats treated with a moderate and high dose of tiger energy drink compared with the control group. The results recorded that significant elevation in the percentage of lymphocytes of rats treated with a moderate and high dose of tiger energy drink compared with the control group. Moreover, the study found that there was a significant (P≥0.05) elevation in total red blood corpuscles, hemoglobin , PCV and MCV of rats treated with a high dose of tiger energy drink compared with the control group. Moreover, the study found that there was a significant (P≥0.05) elevation in total red blood corpuscles, hemoglobin, PCV and MCV of rats treated with high doses of tiger energy drink compared with low doses. The study elucidated that significant increment (P≥0.05) in serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase catalase (ALT) for rats treated with a low moderate and high dose of tiger energy drink compared to the control group. Also, the result of the current study recorded that significant increase (P≥0.05) in serum urea in rats treated with low, moderate and high doses of tiger energy drink compared to the control group.

Keywords: Energy drink, Blood, Liver, Kidney functions.

Introduction

Nowadays, energy drinks consumers especially young individuals are increasing which leads to Caffeine abuse. The term Caffeine is the central nervous system (CNS) that stimulates methyl xanthine class (Reissig et al., 2009). Globally, Caffeine recorded as a most consumed psychoactive drug while it has both positive and negative health effects. In addition, some studies show Caffeine negative health effects such as heart problem, psychiatric problems and reproductive diseases (Quadra et al., 2020). Energy drinks are popular and commonly consumed worldwide particularly by adults aging 35 years and less due to their ability to boast mental and physical performance. There are many kinds of energy drinks such as tiger, red bull and power horse, all these beverages mainly contain caffeine, in addition to water, carbohydrates, vitamins, amino acids and minerals (Marashli, 2021). Excessive consumption of Caffeine, the main active ingredient in energy drinks, by children and adolescents may result to Caffeine intoxication which causes to vomiting, seizures, tachycardia, cardiac arrhythmias, and death (Seifert et al., 2011). Several warnings have been issued from studies regarding possible adverse effects of energy drinks including liver and renal toxicity , showed that the overdose of Caffeine and other bioactive ingredients

in energy drink decrease erythrocytes and WBC counts, HB and PVC value due to its effect in action of hematopoietic system (Olaleru and Odeigah, 2015).

The main mechanism action of Caffeine is focus on the antagonism of adenosine receptors . Thus, the molecular structure of adenosine is like Caffeine, which both have a double bond structure, while Caffeine can occur the adenosine receptor sites , mentioned that administration of ED caused renal toxicity that demonstrated by an elevation of urea, uric acid and creatinine and this elevation was time dependent (Tofovic et al., 2002).

In a healthy body condition, excess substances in energy supplements will be processed first in the liver and then excreted by the body through urine fluid, sweat, or faeces (Utiu et al., 2014).With the presence of stimulant ingredients such as taurine and caffeine found in energy supplements, the liver's work becomes much more difficult. Damage to liver function can be caused by a variety of factors, including a drinking habit, viral infections, and long-term use of certain drugs. People are increasingly suffering from liver damage as a result of their addiction to energy drinks (Stipanuk, 2004).

Materials and Methods

Tiger energy drink: The local Al-Najaf, Iraq market bought the tiger energy drink. The major constituents of these energy drinks are caffeine, guarana, taurine, ginseng, vitamins and carbohydrates. Tiger energy drink (250 ml) of contains Carbonated water, Sugar, Citric Acid, Trisodium citrate, Caffeine 0.03%, Taurine 0.37%, Glucuronolactone 0.24%, B vitamins (B2, B6, B12, Pantothenic Acid, Niacin), Colors (Caramel positive E 150 C), Benzoic Acid and Flavorings.

Animals of experimental: This study was conducted on 12 adult male Wistar Albino rats (Rattus norvegicus), aged 2.5-3 months and weighing 70 - 190 gm., obtained from the animal's house in the Faculty of Science - University of Kufa. The animals were housed in the animal house of the Faculty of Science, University of Kufa, under standard environmental conditions (temperature 25-28 C° and 12 hr light-dark cycle) and allowed access to a standard diet and water, during the period from November 2023 to December 2023. All animal experiments had been approved by the central committee for bioethics at the University of Kufa and were performed according to these guidelines.

Experimental design: The animals were divided into 4 groups of 3 (3 rats/group). The control group (Group 1) rats were maintained on the basal diet and distilled water throughout the experiment. Three levels of tiger energy drink exposure were used: mild, moderate and high tiger energy drink. Rats in the mild tiger energy drink intake group (Group 2) were given a low dose of tiger energy drink (0.3 ml / 100 g body weight/day) to simulate a low human consumption pattern (250 ml/ED can), while the moderate tiger energy drink group (Group 3) received 1.2 ml /100g body weight/day, to reflect moderate human consumption level of 750ml (about 3 cans of ED). The high tiger energy drink intake group (Group 4) was given 4.3 ml/100 g body weight/day to mimic the estimated high human consumption level of 1500 ml (about six cans). All treatments were given by gavage and lasted for four weeks.

Collection of blood samples: Following the end of the experiment, each animal was slightly anaesthetized by (Chloroform 99.5%) (Kavakli et al.,2011). The rats were put after anaesthesia on a dissection bowl, the fore and hind limbs were fixed by fine pins. Heart puncture was done with a 5 ml disposable syringe and 2-5 ml blood was drawn very gently and slowly. Each blood sample was divided into 2 parts. The first part (about 1 ml) was placed in a tube containing EDTA (22mg/ml) as an anticoagulant and mixed thoroughly, then used for the determination of hematological analysis by an automatic analyzer. The remaining blood was placed in the test tube containing gel and left for 30 minutes at room temperature and used to obtain serum via centrifugation at 3000 rpm for 15 minutes to separate serum and put in Epindroff tubes which were kept at 20 in a freezer for determination of biochemical analysis.

Hematological analysis: The hematological parameters were performed on EDTA blood use of an automated auto-analyzer Ruby (Abbott., U.S.A), measurement of the blood parameters includes: total leukocyte count, differential counts of leukocytes, Total RBCs, Hb, PCV, MCV, MCH, MCHC .

Determination of liver and kidney function tests: To assess the state of the liver and kidney, biochemical studies involved analysis of parameters of liver and functions tests such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total protein, serum albumin and total serum Bilirubin with kidney functions tests such as creatinine and urea were analyzed using clinical fully automatic blood chemistry analyzer biochemistry analyzer Ds-261 (Sinnowa medical sciences & technology Co. Ltd., Jiangsu, China).

Statistical Analysis: The data were statistically analyzed by using SPSS (statistical package for social sciences). The independent sample t-test, ANOVA (analysis of variance). All values were expressed as mean ± Standard division. P-value less than 0.05 considered statistically significant.

Results and Discussion

The present study has found that there was a significant (P≥0.05) elevation in total leukocyte count of rats treated with a moderate and high dose of tiger energy drink compared with the control group (Table 1). Also, the study recorded that significant elevation in the percentage of lymphocytes of rats treated with a moderate and high dose of tiger energy drink compared with the control group (Table 1). The results of the study might be explained that elevated WBC count might indicate activation of the immune system, a normal cell–mediated immune response and an increase in lymphocytes and monocytes could be attributed to the action of caffeine that stimulates the hemopoietic system to release more of these cells (Reissig et al., 2009) demonstrated that caffeine treatment during exercise leads to a greater degree of leukocytosis, lymphocytosis and muscle damage.

Table (1) Effect of three doses of tiger energy drink on total and differential counts of leukocytes of rats.

Parameters	Group 1 Control	Rats treated daily with different doses		
		Group 2 Low dose	Group 3 Moderate dose	Group 4 High dose
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Total leukocyte count ($\times10^3$) (cell/mm^3)	6.25±0.38	5.34±0.32a	7.25±0.37ab	9.89±0.39abc
Neutrophils (%)	51.40±2.55	8.40±2.55a	14.40±2.55ab	16.40±2.55abc
Lymphocytes (%)	41.26±2.37	85.40±2.50a	76.25±2.37ab	78.89±2.39abc
Mid-cells (%)	7.34±2.76	8.34±2.72a	8.34±2.70a	16.34±2.74abc

Notes: SD = Standard deviation. (a) = indicate statistically significant (p≥ 0.05) as compared with control. (b) = indicate statistically significant difference as compared with time exposure (30 minutes) at (p≥ 0.05). (c) = indicate statistically significant difference as compared with time exposure (60 minutes) at (p≥ 0.05).

The study found that there was a significant (P≤0.05) elevation in total red blood corpuscles, hemoglobin, PCV and MCV of rats treated with a high dose of tiger energy drink compared with the control group (Table 2). The results of the study might explain that consumption of energy drinks alone or in combination with alcohol is associated with significant alterations in some biochemical parameters. significant changes in RBC value in the present study might be due to changes in the membrane cholesterol and phospholipid content and/or ratio. Erythrocytes in rats treated with Red Bull or Power Horse were hypochromic and showed poikilocytosis and anisocytosis.

Table (2): Effect of three doses of tiger energy drink on rats' total red blood corpuscles and indices.

Parameters	Group 1 Control	Rats treated daily with different doses		
		Group 2 Low dose	Group 3 Moderate dose	Group 4 High dose
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Total RBCs ($\times10^6$) (corpuscle/mm^3)	6.21±0.42	6.14±0.42	5.91±0.41	6.91±0.32ac
Hb (g/dl)	12.54±0.28	11.84±0.24	11.24±0.20	13.34±0.22abc
PCV (%)	38.42±0.14	38.22±0.12	36.42±0.10	42.42±0.16abc
MCV (fL)	51.51±4.49	57.51±4.42	59.51±4.44	61.51±4.48abc
MCH (pg)	19.44±0.71	18.44±0.72	18.34±0.74	19.74±0.78abc
MCHC (g/dl)	31.88±0.82	31.33±0.80	30.63±0.84	31.70±0.86

Notes: SD = Standard deviation. (a) = indicate statistically significant (p≥ 0.05) as compared with control. (b) = indicate statistically significant difference as compared with time exposure (30 minutes) at (p≥ 0.05). (c) = indicate statistically significant difference as compared with time exposure (60 minutes) at (p≥ 0.05).

The study elucidated that significant increment (P≤0.05) in serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase catalase (ALT) for rats treated with a low moderate and high dose of tiger energy drink compared to the control group (Table 3). These results might be explained by that energy drink (ED) induced significant elevations in serum AST, ALT and ALP, creatinine, BUN and uric acid levels. Increases in the blood levels of hepatic enzymes serve as reliable indicators of liver damage by toxic agents. Similar increases have been reported in serum AST, ALT and ALP of rats exposed to caffeinated energy drink EDs (Khayyat et al., 2014).

Table (3): Effect of three doses of tiger energy drink on liver function test of rats.

Parameters	Group 1 Control	Rats treated daily with different doses		
		Group 2 Low dose	Group 3 Moderate dose	Group 4 High dose
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Aspartate aminotransferase (AST) (Units/L)	56.00±6.28	191.00±6.24a	164.00±6.20a	208.00±6.22abc
Alkaline phosphatase ALP (Units/L)	172.00±1.87	404.66±1.80a	282.00±1.82a	384.66±1.84ac
Alanine aminotransferase catalase (ALT) (Units/L)	34.80±3.43	44.33±3.46a	35.80±3.42a	43.80±3.40ac
Serum Total Protein (g/dl)	7.6±0.11	5.38±0.10a	5.57±0.14a	6.36±0.16abc
Serum Albumin (g/dl)	4.52±0.03	3.42±0.06	3.12±0.04	3.32±0.02a

Notes: SD = Standard deviation. (a) = indicate statistically significant (p≥ 0.05) as compared with control. (b) = indicate statistically significant difference as compared with time exposure (30 minutes) at (p≥ 0.05). (c) = indicate statistically significant difference as compared with time exposure (60 minutes) at (p≥ 0.05).

Energy drink ED demonstrated a toxic effect on liver tissue evidenced by a significant increase in AST. Energy drink brands with low constituents known for antioxidant potential may have deleterious synergistic effects on hepatic status. . have reported that adverse reactions and toxicity from high-energy drinks stem primarily from caffeine content. In support, studies suggest that caffeine administration significantly increased AST, ALT and creatinine in the serum of normal and obese diabetic rats (Mora-Rodriguez & Pallares2014).

The study showed no significant change in the average serum creatinine electrical in rats treated with low, moderate and high doses of tiger energy drink compared to the control group. Also, the result of the current study recorded that significant increase (P≤0.05) in serum urea in rats treated with low, moderate and high doses of tiger energy drink compared to the control group (Table 4). The result of this study might be attributed that increases in blood levels of creatinine are usually associated with impaired kidney function. Several warnings have been issued regarding the potential adverse effects of energy drinks including hepatotoxicity and nephrotoxicity , alterations in the cardiovascular system . and changes in the structure and function of secretory glands (Heckman et al.,2010).

Qassim et al., (2022) found that giving a low dose of energy drink leads to mild to moderate renal damage, whereas, high doses result in severe damage. Moreover, in humans, cases of kidney injuries were reported following the consumption of these drinks . Thus it is indicated that energy drinks are nephrotoxic on chronic consumption and their toxicity is dose-dependent.

Conclusions

This study found that high concentrations consumption of tiger energy drink could cause a significant elevation in total leukocyte count, percentage of lymphocytes of rats. This study confirms that treating rats with high doses of tiger energy drink for 30 days leads to negative effects on the liver and kidneys.

Table (4): Effect of three doses of tiger energy drinks on kidney function test of rats.

Parameters	Group 1 Control	Rats treated daily with different doses		
		Group 2 Low dose	Group 3 Moderate dose	Group 4 High dose
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Serum creatinine (mg/dl)	0.76±0.08	0.56±0.04	0.62±0.06	0.66±0.02
Serum urea (mg/dl)	28.20±4.92	47.66±4.96a	66.66±4.94ab	62.20±4.90ac

Notes: SD = Standard deviation. (a) = indicate statistically significant (p≥ 0.05) as compared with control. (b) = indicate statistically significant difference as compared with time exposure (30 minutes) at (p≥ 0.05). (c) = indicate statistically significant difference as compared with time exposure (60 minutes) at (p≥ 0.05).

References

Reissig, C. J., Strain, E. C., & Griffiths, R. R. (2009). Caffeinated energy drinks—a growing problem. Drug and alcohol dependence, 99(1-3), 1-10.

Quadra, G. R., Paranaíba, J. R., Vilas-Boas, J., Roland, F., Amado, A. M., Barros, N., ... & Cardoso, S. J. (2020). A global trend of caffeine consumption over time and related-environmental impacts. Environmental Pollution, 256, 113343.

Marashli, L. T. (2021). The Difference in Caffeine Consumption among College Students at the Beginning and End of the Semester (Master's thesis, Kent State University).

Seifert, S. M., Schaechter, J. L., Hershorin, E. R., & Lipshultz, S. E. (2011). Health effects of energy drinks on children, adolescents, and young adults. Pediatrics, 127(3), 511-528.

Olaleru F, Odeigah PGC (2015). Effects of Energy Drink on Sperm Morphology, Haematological Parametres and Behaviour of Adult Male Mice. Annual Res. Rev. Biol. 6: 288-296.

Tofovic, S.P., Kost Jr., C.K., Jackson, E.K. and Bastacky, S.I. (2002). Long-Term Caffeine Consumption Exacerbates Renal Failure in Obese, Diabetic, ZSF1 (fa-facp) Rats. Kidney International, 61, 1433-1444.

Utiu,I I., Rosioru,C.L.,Lang,C. and Munteanu,C.(2014). Chronic administration of red bull affects blood parameters in rats. Studia Universitatis Babes-Bolyai, Biologia, 59(2).

Stipanuk, M. H. (2004). Role of liver in the regulation of body cysteine and taurine levels: a brief review. Neurochem Res. 29:105–10.

Reissig, C. J., Strain, E. C., & Griffiths, R. R. (2009). Caffeinated energy drinks—a growing problem. Drug and alcohol dependence, 99(1-3), 1-10.

Khayyat, L. I., Essawy, A. E., Al Rawy, M. M., & Sorour, J. M. (2014). Comparative study on the effect of energy drinks on haematopoietic system in Wistar albino rats. Journal of environmental biology, 35(5), 883.

Heckman, M. A., Sherry, K., & De Mejia, E. G. (2010). Energy drinks: an assessment of their market size, consumer demographics, ingredient profile, functionality, and regulations in the United States. Comprehensive Reviews in food science and food safety, 9(3), 303-317.

Kavak, S., Meral, I., Pirinçci, N., Güneş, M., Demir, H., ... & Ceylan,K. (2011). Effects of shock waves on oxidative stress, antioxidant enzyme and element levels in kidney of rats. Biological trace element research, 144(1-3), 1069-1076.

Mora-Rodriguez, R., & Pallares, J. G. (2014). Performance outcomes and unwanted side effects associated with energy drinks. Nutrition reviews, 72(suppl_1), 108-120.

Qassim, A. H., Alsammak, M. A., & Ayoob, A. A. (2022). Histopathological changes in kidney and pancreas induced by energy drinks in adult male rats. Iraqi Journal of Veterinary Sciences, 36(1), 111-116.