https://doi.org/10.37939/jrmc.v28i3.2702

Implications Of Ingesting Soft Drink And Energy Drink On Hepatic Microcirculation Of Albino Rat

Saira Munawar[1], Faiza Irshad[2], G.P. William[3], Muhammad Suhail[4], Ayesha Yousaf[5], Ahmad Haris Suhail[6]

Abstract

Objective: To compare the diameter of the centrilobular vein as well as its congestion along with that of sinusoids and portal vein of the rat liver after ingestion of caffeinated soft drinks and energy drinks which were given caffeinated soft drinks and energy drinks.

Method: 11ml/kg body weight each, of distilled water to the control group, caffeinated soft drink to the SD group and caffeinated energy drink to the ED group was given respectively for 8 weeks. Later, the rats were sacrificed, the liver dissected out, slides prepared and stained with H & E for microscopic observation.

Result: Morphometric analysis showed an increase in the diameters of centrilobular veins of both experimental groups receiving caffeinated SD and ED compared to control group p-values <0.001. The SD group was affected more (p-value 0.024)

Conclusion: Caffeine-containing ED and SD are held responsible for congestion and dilatation of centrilobular veins, portal veins and sinusoids of albino rats, the augmented effect seen with SD could be because the high taurine content of ED would have interfered with the metabolism of the caffeine.

MeSH Keywords: Energy drink, Soft drink, Caffeine, Taurine, Liver, Portal vein.

[1] Associate Professor of Anatomy, Fatima Jinnah Medical University, Lahore; [2] Associate Professor of Anatomy, Azra Naheed Medical College, Lahore; [3] Associate Professor of Anatomy, FMH College of Medicine & Dentistry, Lahore; [4] Head of Department of Anatomy, Amna Inayat Medical College, Sheikhupura; [5] Head of Department of Anatomy, Rawalpindi Medical University; [6] Consultant Anaesthetist, Integrated Medical Centre, Lahore.

Correspondence: Dr. Saira Munawar, Associate Professor of Anatomy, Fatima Jinnah Medical University, Lahore. **Email:** drsairamunawar@gmail.com

Cite this Article: Munawar S, Irshad F, William G, Suhail M, Yousaf A, Suhail AH. Implications Of Ingesting Soft Drink And Energy Drink On Hepatic Microcirculation Of Albino Rat. JRMC. 2024 Sep. 30;28(3).522-527. https://doi.org/10.37939/jrmc.v28i3.2702.

Received March 19, 2024; accepted August 27, 2024; published online September 26, 2024

1. Introduction

Both energy drinks (ED) and soft drinks (SD) are subcategories of nonsteroidal, non-alcoholic caffeinated beverages that are equally popular among teenagers as well as adults, owing to various energy-boosting ingredients like taurine and caffeine which help them with their focus, concentration, alertness and physical performance. Coca-Cola is a widely used caffeinated soft drink, having 34mg of caffeine / 350 ml on the other hand, Red Bull is the preferred choice of energy drinks with more than double the amount of caffeine and contains many legal stimulants other than taurine.[1] Their consumption is consistently on the rise, affecting both the developed as well as the underdeveloped countries across the globe.[2] Although energy drinks are expensive, and higher in their caffeine content than soft drinks, both drinks are in trend due to 360◦ marketing strategies and accessibility. FDA considers soft drinks and energy drinks as conventional /functional food thereby poorly regulating them. Even then Energy drinks (ED) faced a few inconsistent bans, due to their potential toxicity in various European countries.[3]

The adverse effects of the caffeine content of ED drinks have also been reported on digestive, renal, neurological and reproductive health.[4] High quantities of taurine are added to ED, as they are also held responsible for enhancing the physical performance of the consumer by increasing the blood flow to various viscera but at the same time, its adverse effects on the cardiovascular system,[1] kidney and liver are also reported.[5] Caffeinated soft drinks, which contain caffeine only and not another stimulant, were held responsible for increased plasma triglycerides, lipogenesis, and fatty liver. Daily consumption of caffeinated soft drinks for longer duration may elevate the risk of weight gain, metabolic syndrome, inflammation and altered hepatic architecture.[6] Besides affecting the gastrointestinal tract, and consumption of energy drinks, more than 50% of adverse effects, are on the cardiovascular system including hypertension, myocardial infarctions, arrhythmia and sudden death.[4]

Due to their skyrocketing consumption, lack of sufficient evidence of their toxicity, and occasional acute adverse health effects, the safety of these drinks is dubious.

The liver is crucial for metabolizing most constituents of soft drinks as well as energy drinks into easily excreted metabolites and is therefore vulnerable to toxicity, which is influenced by the daily intake of these drinks.[7] The hepatic lobule is the classical structural and functional unit of the liver and its microcirculation. The hepatic microcirculation begins

with the portal venule that drains to the hepatic sinusoid and then to the centrilobular veins. Under the light microscope, the hepatic lobules are hexagonal with terminal hepatic venule, also known as the centrilobular vein in the center, and portal venule within the portal triad, at each corner of the hexagon. The sinusoids radiate from the portal venule to the central vein, whereas the plates of hepatocytes are separated from each other by sinusoids.[8] The function of endothelial cells lining vasculature declines with the caffeine and sugar content of these drinks.[9] Therefore, most adverse effects are expected in the liver as well as the vascular system.

Most literature cites the adverse effects of energy drinks or soft drinks individually on animals or humans, a few studies showed that the results of human and animal experimental studies overlap in this regard.[4] Scarce to no data is available comparing the effects of caffeinated soft drinks and energy drinks on hepatic vasculature, so this study was designed to evaluate the dilatation and congestion of the centrilobular vein by measuring the diameter of the centrilobular vein and state of congestion of hepatic sinusoids and portal triads, comparing the effects of caffeinated soft drinks and energy drinks on liver microcirculation.

2. Materials & Methods

The experimental study was conducted on 45 albino rats at the Federal Postgraduate Medical Institute (FPGMI), Lahore, after ethical clearance from the university. The experiment was conducted according to the guidelines for the care of laboratory animals, food and water were freely available to the rats, while the room temperature and 12-hour dark/ light cycle were also maintained in the room of the animal house throughout the experiment. Once acclimatized, 15 albino rats were randomly distributed to each of the three groups namely Control, SD (soft drink) and ED (energy drink). Control Group, SD group (experimental group) and ED group (experimental group) were given a dose of 11ml/kg body weight/day of distilled water, soft drink (Coca-Cola) and energy drink (Red Bull) respectively for 8 weeks. The dose calculated was equivalent to the ingestion of 750 ml of these drinks by a 70 kg man.[10] At the end of the experiment the rats were euthanized, and dissection was done to isolate the livers, which were washed with normal saline and then fixed with formalin. Sections measuring 0.2 cm × 0.2 cm were taken from each lobe of the livers along with any additional area of visible abnormality.[11] By using the automated tissue processor, the sections were dehydrated (graded solutions of alcohol), cleared with xylene, infiltrated and embedded by paraffin and tissue blocks were formed. Later, 5um thin tissue ribbons were cut by using a rotary microtome and a slide was prepared and stained with hematoxylin and eosin.

The control group was compared with experimental groups for the presence of congestion in hepatic sinusoids, portal venules and centrilobular veins. The micrometre of the objective as well as the eyepiece was superimposed, for measuring the diameter of the centrilobular vein. For calculating the diameter of the centrilobular vein both the transverse and vertical diameters were measured and then their average was calculated. (Figure 1)

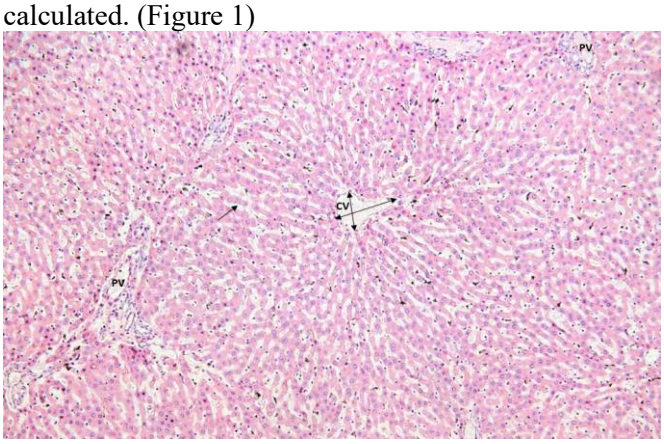

Figure 1: Control group at 10 x, centrilobular vein represented by CV, portal vein by PV & hepatic sinusoids by black arrow. Measurement of the diameter of a centrilobular vein (CV) from one edge to the other

Five different fields were observed to calculate the diameter of the centrilobular vein, and then the mean was calculated. Chi-square and one-way ANOVA were applied to analyze the data using the statistical package for social sciences (SPSS 22.0). One-way ANOVA was followed by Tukey's test for comparison among various groups.

3. Results

1. Congestion of Centrilobular Vein

Dilated centrilobular veins having red blood cells (RBC) are known as congested centrilobular veins. Significant congestion (Figure 2) was found in the centrilobular veins SD group (87%) and ED (80%) group in comparison to a control group with a p-value <0.001. Results are tabulated in the table. 1. However, both the

experimental groups were similarly congested with no statistical significance p- value 0.624. (Figure 3)

2. Congested Portal Triads:

Portal triads are said to be congested when the tributaries of the veins in portal triad are dilated and filled with RBC. (Figure 2) Portal triads of 80% of the livers of rats of the SD group and 73% of the ED group are congested as compared to a control group with a p-value of <0.001 which is significant. But the congestion in both experimental groups was statistically insignificant with a p-value of 0.666. (Figure 3)

3. Congestion in Hepatic Sinusoids:

The presence of red blood cells (RBC) in hepatic sinusoids is labelled as vascular congestion (Figure 2) and was observed in (60% of) the livers of in SD group and (53% of) the livers of the in the ED group in contrast to the control group (p-value 0.001), which was statistically significant, but the SD and ED groups were not significantly different from each other p-value 0.713 (Figure 3)

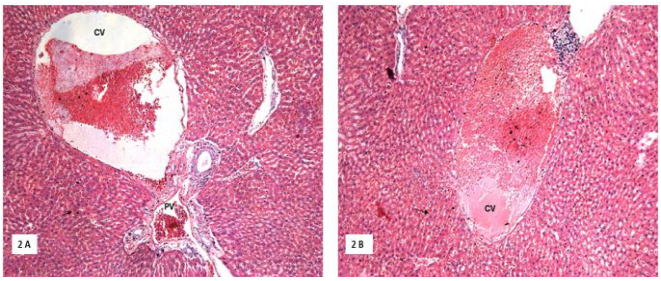

Figure 2A: SD group at 10 x, Congested (RBC's)centrilobular vein represented by CV, congested (RBC's) portal vein by PV & congested (RBC's)hepatic sinusoids by black arrow. 2B ED group at 10 x, Congested centrilobular vein represented by CV & congested hepatic sinusoids by black arrow

Table 1: Showing the results of various parameters in the SD as well as ED group in comparison to the Control

Histological Parameters	Control	SD Group	ED Group
Congestion in Centrilobular Veins	0%	87%	80%
Congestion in Hepatic Sinusoids	0%	60%	83%
Inflammation in Portal Triads	0%	87%	80%
Congestion in Portal Triads	0%	80%	73%

4. Diameter Of Centrilobular Vein:

The mean diameters of the centrilobular vein for; the control group were 51.9 ± 5.5 µm, the SD group was 141.6± 37.7 µm, and the ED group was 113 ± 31.5 µm, which means the diameter of the SD and ED groups were significantly more than the control group (p value 0.001). Also, the diameter of the SD group is substantially greater than ED group (p-value 0.024).

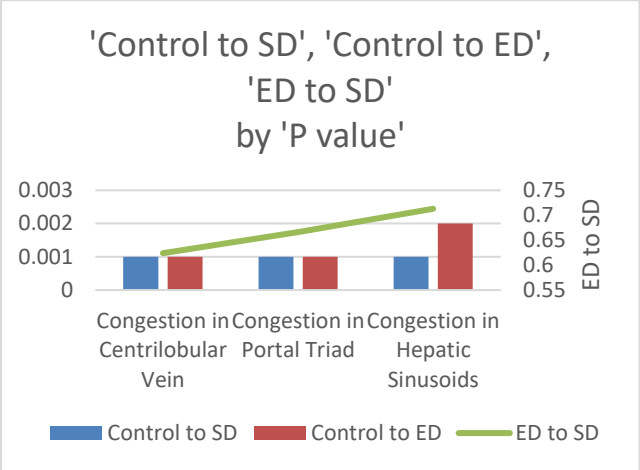

Figure 3: Showing the comparison of control with SD and ED as well as SD to ED for Congestion in centrilobular vein, hepatic sinusoids & portal vein, blue represents control to SD, p-value which is <0.001 in cases of congestion centrilobular vein, portal triad and hepatic sinusoid and is statistically significant, whereas maroon represents control to ED, p-value which is <0.001 in cases of congestion centrilobular vein, portal triad and hepatic sinusoid and is also statistically significant. The green line represents a comparison of the p-value of SD and ED, which in the case of congestion of the centrilobular vein is 0.624, the portal triad is 0.666, and the hepatic sinusoid is 0.713 which is statistically insignificant.

4. Discussion

This study demonstrates that oral consumption of SD and ED for 8 weeks by albino rats has adversely affected the liver causing congestion and dilatation in the hepatic microcirculation. Globally, the younger population is consuming various soft drinks and energy drinks at an alarming rate and frequency, adding to the disease burden of both developed and developing countries across the world.[2]

These drinks are not only responsible for increasing the risk of obesity, hyperlipidemia, metabolic syndrome, liver and kidney damage but also acute cardiovascular events and sudden deaths.[4]

Most energy drinks have 80-150mg of caffeine in 350 ml and half of this quantity is present in soft drinks.[1] It does enhance physical and mental performance but increases the risk of psychosis and seizures but during physical exercise, it blocks the adenosine receptors thereby causing sluggish blood flow to the heart and reducing the diameter of coronary vessels.[12] On the other

hand taurine, 2-aminoethane-sulfonic acid, is present in human tissues and can be synthesized endogenously by the liver using cysteine. It is therapeutically used for reducing inflammation, cardiac events, and seizures. It is known for its antioxidant, antitumor and antifibrotic properties but is also implicated in weight reduction and dehydration.[13] Taurine exerts its hepatoprotective role by inhibiting the production of reactive oxygen species.[1]

In the present study, both the experimental groups SD and ED had congested centrilobular veins, portal veins, and hepatic sinusoids, which implies that SD and ED have equally affected the liver's microcirculation. Several studies support the findings of our study, but those studies focus on the effects of SD or ED and do not compare the effects of SD and ED on the liver, which was the aim of this study.

Samia Ali al-askalany et al. reported congested centrilobular veins in the livers of the rats which were given cola drink for 8 weeks.[14] Alkhedaide et al. gave coca-cola to Wistar rats for 12 weeks, which resulted in congested blood vessels in their liver and kidney.[15] Our findings coincide with another study in which congestion of blood vessels in the livers of rats treated with different kinds of energy drinks was observed by Khayyat et al.[16] A study done by Bukhar et al. also reported dilation and congestion of sinusoids as well as portal vessels in rats.[17] Salih et al. reported congested hepatic sinusoids of the rabbit after receiving Red Bull energy drink for 4 weeks, which is consistent with our findings.[18]

In this study, the centrilobular veins of both the ISD & ED groups were dilated and had greater diameter than the control group. Moreover, the SD group had larger centrilobular veins not only in comparison to the control but also ED group. Nahla E. Ibrahem also reported dilated centrilobular veins of the albino rats when given a Red Bull energy drink for 4 weeks.[5] Salih et al. also observed dilated centrilobular veins in rabbit liver with Red Bull energy drink.[18]

SD and ED contain caffeine which has increased the diameter of the centrilobular vein. This effect may have been a bit reduced in the ED group because of the concomitant presence of taurine (hepatoprotective) along with caffeine (hepatotoxic) instead of just caffeine in the SD group. Rabab Mubarak also reported dilated blood vessels in an experiment on the submandibular glands of rats receiving Red Bull.[19] This is supported by the work of Bukhar et al., who showed dilated hepatic and portal blood vessels in rats receiving energy drinks.[17]

This dilatation and congestion of the centrilobular vein, portal vein and hepatic sinusoids can be because of the main ingredient, “caffeine” present both in SD and ED. Caffeine can cause vascular dilatation by two pathways, either directly by increasing the cAMP in smooth muscles of the blood vessels, by competitively inhibiting the phosphodiesterase, or indirectly by raising intracellular calcium, which results in the release of nitric oxide synthase in endothelial cells, releasing nitric oxide and dilation of the vessels. Removal of stimulus for smooth muscle contraction along with amplification in relaxation of smooth muscles of vessels leads to vasodilation. Another mechanism of action of caffeine is that it acts directly on the endothelial cell, stimulates the production of nitric oxide and hence vasodilation.[20]

Numerous studies suggest the decreased endothelial function of the blood vessels especially coronary vessels with the consumption of energy drinks. It may be due to caffeine, or other stimulants like taurine and glucuronolactone or their combined effect. Endothelial dysfunction due to ED may be associated with a raised risk of myocardial infarction implicating the decreased endothelial cell function.[9] Caffeine has been held responsible for amplifying oxidative stress in vessels through the enhanced quantity of angiotensin II, by preventing the influence of adenosine on the renin-angiotensin system.[20] Caffeine ingestion can affect the cardiovascular response to exercise.[12]

Hence, caffeinated energy drinks (ED) and soft drinks (SD) caused congestion and dilation in the centrilobular veins, portal veins, and sinusoids of albino rats, with a more pronounced effect from soft drinks. Although taurine is considered hepatoprotective, its presence even in large quantities along with caffeine in the energy drink could not prevent congestion in the hepatic microcirculation of the present study. This may be because taurine behaves differently in the presence of caffeine. [1,19]

Given the higher morbidity and mortality rates associated with the consumption of caffeinated energy and soft drinks in younger populations, there is a critical need for increased public awareness. Additionally, restrictions on the availability and marketing of these beverages should be considered, with regulations aiming to reduce their potential negative impact on human health.

More research is suggested on several types of commercially available, regular as well as diet, caffeinated soft and energy drinks and their possible histological effects on various organs. Also to evaluate, if consumption of these drinks is discontinued then, the effects will be reversed or not?

5. Conclusion

Therefore, caffeine-containing ED and SD are held responsible for congestion and dilatation of centrilobular veins, portal veins and sinusoids of albino rats, the augmented effect seen with SD could be because the high taurine content of ED would have interfered with the metabolism of the caffeine. Owing to the potential risk emphasized by our study, it is prudent to be cautious regarding the consumption of these drinks.

INSTITUTIONAL REVIEW BOARD

00291116MMANA Dated 30-11-2016

CONFLICTS OF INTEREST- None

Financial support: None to report.
Potential competing interests: None to report
Contributions:
S.M, M.S, A.Y - Conception of study
S.M - Experimentation/Study Conduction
S.M, F.I, G.P.W, A.H - Analysis/Interpretation/Discussion
S.M, F.I, G.P.W - Manuscript Writing
R.N.K, S.B.A, M.L, M.S, A.Y, A.H - Critical Review

All authors approved the final version to be published & agreed to be accountable for all aspects of the work.

References

1. Wassef B, Kohansieh M, Makaryus AN. Effects of energy drinks on the cardiovascular system. World journal of cardiology. 2017;9(11):796. doi: 10.4330/wjc.v9.i11.796
2. Nadeem IM, Shanmugaraj A, Sakha S, Horner NS, Ayeni OR, Khan M. Energy drinks and their adverse health effects: a systematic review and meta-analysis. Sports health. 2021;13(3):265-77. https://doi.org/10.1177/1941738120949181
3. Mulhern MP, Sinha MS. Labeling Energy Drinks: Tackling a Monster of a Problem. Saint Louis U Legal Studies Research Paper. 2024(2024-05). https://ssrn.com/abstract=4782029. https://doi.org/10.2139/ssrn.4593238
4. Costantino A, Maiese A, Lazzari J, Casula C, Turillazzi E, Frati P, et al. The dark side of energy drinks: a comprehensive review of their impact on the human body. Nutrients. 2023;15(18):3922. https://doi.org/10.3390/nu15183922
5. Ibrahem N, Reda S, Mekawy N. Hepatic Changes under the Effect of Red Bull Energy Drinks and its Withdrawal in Adult Male Albino Rats (Histological and Immunohistochemical Study). Journal of Medical Histology. 2022;6(1):34-43. https://dx.doi.org/10.21608/jmh.2022.170936.1106
6. Malik VS, Hu FB. The role of sugar-sweetened beverages in the global epidemics of obesity and chronic diseases. Nature Reviews Endocrinology. 2022;18(4):205-18. DOI: 10.1038/s41574-021-00627-6
7. Ilesanmi OB, Odewale TT. Effect of classic soft drink Coca-Cola as a solvent in the administration of tramadol and diazepam on biochemical and histological changes in liver and kidney. Ukrainian Journal of Nephrology and Dialysis. 2020.67 (3):33-41. https://doi.org/10.31450/ukrjnd.3(67).2020.06
8. Kan Z, Madoff DC, editors. Liver anatomy: microcirculation of the liver. Seminars in interventional radiology; 2008: © Thieme Medical Publishers. https://doi.org/10.1055%2Fs-2008-1076685
9. Higgins JP, Liras GN, Liras IN, Jacob R, Husain F, Pabba KC, et al. Energy drink effects on hemodynamics and endothelial function in young adults. Cardiology. 2021;146(2):258-62. https://doi.org/10.1159/000512433
10. Ferreira SE, Quadros IMH, Trindade ÁA, Takahashi S, Koyama RG, Souza-Formigoni MLO. Can energy drinks reduce the depressor effect of ethanol? An experimental study in mice. Physiology & Behavior. 2004;82(5):841-7. https://doi.org/10.1016/j.physbeh.2004.06.017
11. Avwioro G, Iyiola S, Aghoghovwia B. Histological and biochemical markers of the liver of Wistar rats on subchronic oral administration of green tea. North American Journal of Medical Sciences. 2010;2(8):376. https://doi.org/10.4297%2Fnajms.2010.2376
12. Harber MP, McCurry A, Carlini N, Kistler B, Fleenor BS. Caffeine ingestion alters central hemodynamics following aerobic exercise in middle-aged men. European Journal of Applied Physiology. 2021;121:435-43. https://doi.org/10.1007/s00421-020-04521-3
13. De Luca A, Pierno S, Camerino DC. Taurine: the appeal of a safe amino acid for skeletal muscle disorders. Journal of translational medicine. 2015;13:1-18. https://doi.org/10.1186/s12967-015-0610-1
14. Al-Askalany Sa, Sadek Mm, Mohamed Nm. The effects of carbonated soft drinks on some biochemical blood parameter and liver histopathology of experimental rats. Egyptian Journal of Agricultural Research. 2018;96(1):97-109. https://dx.doi.org/10.21608/ejar.2018.130411
15. Alkhedaide A, Soliman MM, Salah-Eldin A-E, Ismail TA, Alshehiri ZS, Attia HF. Chronic effects of soft drink consumption on the health state of Wistar rats: A biochemical, genetic and histopathological study. Molecular medicine reports. 2016;13(6):5109-17. https://doi.org/10.3892%2Fmmr.2016.5199
16. Khayyat L, Sorour JMA, Essawy A, Al Rawi M. Histological, ultrastructural and physiological studies on the effect of different kinds of energy drinks on the liver of Wistar albino rat. International Journal of Research in Science. 2015;1(2):15-22. http://dx.doi.org/10.24178/ijrs.2015.1.2.15

17. Bukhar HM, ElSawy NA, Header EA. Biological effect of high energy drink on normal and hyperglycemic rats. Pakistan Journal of nutrition. 2012;11(4):301. https://doi.org/10.3923/pjn.2012.301.309
18. Salih NA, Abdul-Sadaand IH, Abdulrahman NR. Histopathological effect of energy drinks (red bull) on brain, liver, kidney, and heart in rabbits. Medical Journal of Babylon. 2018;15(1):16-20. http://dx.doi.org/10.4103/MJBL.MJBL_5_18
19. Mubarak R. Effect of red bull energy drink on rats submandibular salivary glands (light and electron microscopic study). J Am Sci. 2012;8(1):366-72. http://www.americanscience.org. https://doi.org/10.1016/j.identj.2024.07.022
20. Song X, Kirtipal N, Lee S, Malý P, Bharadwaj S. Current therapeutic targets and multifaceted physiological impacts of caffeine. Phytotherapy Research. 2023;37(12):5558-98. https://doi.org/10.1002/ptr.8000.