

EFFECTS OF CAFFEINATED SOFT DRINKS & ENERGY DRINKS ON ADULT RAT BODY WEIGHT, LIVER WEIGHT AND RELATIVE TISSUE WEIGHT INDEX

SAIRA MUNAWAR¹, SHAGUFTA NASREEN², KANWAL SHARIF³, MUHAMMAD SUHAIL⁴

¹Department of Anatomy, Fatima Jinnah Medical University, Lahore, ²Department of Anatomy, PGMI/AMC, Lahore,

³Department of Anatomy, Azra Naheed Medical College, Lahore,

⁴Department of Anatomy, Shaikh Zayed PGMI, Lahore.

ABSTRACT

Objective/Background: Youngsters are consuming caffeinated soft drinks and energy drinks at alarming rates without knowing their safety. Hence, the research was conducted to observe the effects of most universally used caffeinated soft drink (SD), (Coca Cola) and energy drink (ED) (Red Bull) on the body weight and liver of rats.

Methods: Control group, SD group (experimental group) and ED group (experimental group) were given a dose of 11ml/kg body weight of distilled water, Coca Cola and Red Bull per day, respectively for 8 weeks. At the start and at the end of the experiment, all the rats were weighed and then the rats were dissected, and their livers were weighed and Relative tissue weight index (RTWI) calculated.

Results: The gain in body weight of both experimental groups at the end of the experiment was insignificant in comparison to the control group. The liver weight of experimental groups was more than the control group yet it was not statically significant but SD group had a higher RTWI in comparison to the control group.

Conclusion: Consumption of Caffeinated soft drinks (Coca Cola) and energy drinks (Red Bull) raised body weight but the liver weight remains almost unaffected in comparison to control whereas RTWI of SD group was significantly more. Caffeine content of both these could be held responsible for these affects, thereby making their consumption questionable. The dose of 11ml/kg body weight given to rats in this study equals to consumption of 3 cans of these drinks by 70 kg man, hence consumption of these drinks require caution as they affect the weight of liver and relative tissue weight index.

Key words: Soft drinks, RTWI, caffeine.

How to cite this article: Munawar S, Nasreen S, Sharif K, Suhail M. Effects of caffeinated soft drinks & energy drinks on adult rat body weight, liver weight and relative tissue weight index. *Pak Postgrad Med J* 2019;30(2): 82-86.

INTRODUCTION:

Caffeinated, nonalcoholic, artificially sweetened drinks are marketed as soft drinks (SD) and energy drinks (ED). Energy drinks have more caffeine than soft drinks, in addition they also have taurine and many other energy boosting constituents.¹ The target population of both soft drinks as well as energy drinks is teenagers and young adults. Although energy drinks are expensive in comparison to cola soft drinks, but enticing marketing strategies, easy availability and peer pressure or its usage as a status symbol and for other

recreational purposes have led to greater consumption of both these drinks over the past few years.

On average, students consume up to 4 cans of energy drinks in a month, according to them it increased their performance in exams, improved their concentration, helping them to finish their projects and improved physical activity in sports.² Coca Cola is considered to be the most prevalent caffeinated soft drink, and contains caffeine, sucrose (fructose and glucose), caramel coloring, lime and coca extract, phosphoric acid, citric acid, cinnamon, nutmeg, vanilla and orange.³ While Red Bull, is most frequently used energy drink, containing sparkling water, caffeine, glucuronolactone, inositol, niacin, citric acid, pantenol, vitamin B series and taurine.⁴ The quantity of caffeine as well as sugar is by and large the same in most energy drinks.²

Caffeine is a methyl xanthine derivative, found in chocolates, dietary supplements and many beverages

Correspondence to: Saira Munawar, Department of Anatomy, Fatima Jinnah Medical University, Lahore
E-mail: dr.amna22@yahoo.com

Received: July 18, 2020

Revised: September 7, 2020

Accepted: October 6, 2020

including coffee tea, cola soft drinks and energy drinks. It is also present in one of the most accessible, non-prescribed drug throughout the world. Caffeine acts by inhibiting phosphodiesterase enzyme and by acting as an antagonist to adenosine receptor. On an average, an American consumes in excess of 230mg daily; similarly, it is estimated to be 76mg daily in the rest of the world.⁵ LD50 of caffeine in humans is most probably 150-250mg but this varies according to the degree a person is sensitive to it. A case report showed 57mg/kg caffeine was sufficient to be lethal. Literature shows that around 1 g per kg of its intake may start showing its toxic effects but 10 to 14 g/kg has been considered lethal.⁶ It heightens physical and mental performance.⁷ It is held responsible for slowing down the flow of blood to heart during exercise and cause constriction of coronary vessels by blocking adenosine receptors.⁸ Caffeine is also used as a therapeutic agent in premature infants for treatment of apnea & bronchopulmonary dysplasia owing to its bronchodilator effects.⁹ Its consumption in greater quantities is associated with hypertension, hypokalemia, and palpitations. It also has a positive inotropic effect.⁵ Its consumption in doses in excess of 300 mg per kg causes, anxiety, panic and person may hallucinate and can have negative effects on motor skills.¹⁰

Caffeine ingestion during pregnancy, increases the chances of miscarriages, intrauterine growth restriction and low birth weight.^{11,12}

Taurine is 2-aminoethane-sulfonic acid, an amino acid present in ample quantities in human tissues especially liver. Average daily intake amounts to be 60mg.¹³ Human liver can also synthesize it by various enzymes using cysteine as the raw material. Intake of various nutrients especially protein and availability of cysteine affects its endogenous synthesis. It is thought that premature babies are deficient in enzymes responsible for conversion of cysteine from cystathionine, consequently taurine manufactured, may be insufficient. If the babies are not breast fed, they may be deficient in taurine, therefore formula milks have added taurine in them. It is responsible for stabilizing membranes, maintaining calcium homeostasis and regulating ion channels as well as metabolism of muscles. It is known to have osmoregulatory effect and also reduces inflammation and stress of endoplasmic reticulum and aging. It can reprogram and influence gene expression in several tissues including liver and skeletal muscles.¹⁴ Literature shows various therapeutic uses of taurine in humans for example in treatment of diabetes,¹⁵ alcohol withdrawal syndrome, several cardiac disease and seizures.¹⁶ It was also proved beneficial for reducing

inflammation,¹⁷ hepatic disorders¹⁸ and mitochondrial diseases.¹⁶

Researchers indicated that SD intake raised plasma triglyceride, caused lipogenesis, and fatty liver.¹⁹ Mammary gland and pancreatic carcinoma were also witnessed with their consumption.²⁰ Daily SD consumption may also increase the risk of weight gain, metabolic syndrome, inflammation¹ and hepatocellular carcinoma.²¹

Initially, ED were banned in many countries especially in Europe owing to its suspected cardiovascular effects, but later the restrictions were lifted but now frequent emergency room visits of its consumers and the deleterious health effects caused by these drinks has again jolted the authorities to take the necessary action.² Hepatitis, myocardial infarction, gastrointestinal upsets and sudden death were also reported with intake of ED.^{22,23}

METHODS:

Following acclimatization, 15 albino rats were allocated to each group namely Control, SD (soft drink) and ED (energy drink) by using random sampling technique. Rats of all three groups were weighed before the commencement of the experiment as well as at the end of the experiment. Control Group, SD group (experimental group) and ED group (experimental group) were given a dose of 11ml/kg body weight of distilled water, Coca Cola and Red Bull per day, respectively for 8 weeks. Later, the rats were dissected and their livers were weighed and RTWI was calculated. SPSS (22.0) was used to analyze the data.

$$RTWI = \frac{\text{Mean weight of liver}}{\text{Mean body weight of Animal}} \times 100$$

RESULTS:

1. Weight of the Rats (g):

Before the start of experiment, average body weight of Albino rats of control group was 231 ± 44 grams (g). Rats of SD group weighed 224 ± 29 g while those of ED weighed 227 ± 31 g. The statistical difference among these three groups was calculated by applying one-way ANOVA and the difference was insignificant with p-value 0.871. (Fig.1)

After 8 weeks, average body weight of Albino rats of control group increased from 231 ± 44 g to 258 ± 52 g, of SD group from 224 ± 29 g to 264 ± 35 g while those of ED group from 227 ± 31 g to 262 ± 36 g. The statistical difference between these three groups was insignificant with p-value 0.920. (Fig.1) This means that the body weight of rats in SD as well as ED group increased but not significantly in comparison to the control group.

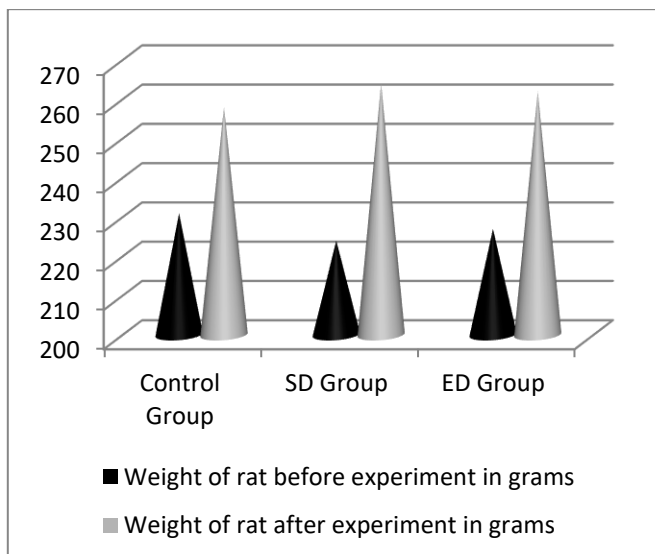


Figure.1: Average body weight of rats of control group, SD group and ED group before and after experiment.

2. Liver Weight of Rats (g):

Average weight of livers of rats of the control group was 10.19 ± 1.88 g, SD group livers weighed 11.67 ± 1.99 g while those of ED group weighed 10.85 ± 1.95 g. This means the weight of livers of SD group were higher than ED group and control group but not statistically significant, p-value 0.126. The ED group had a slight increase in liver weight but not of statistical significance.

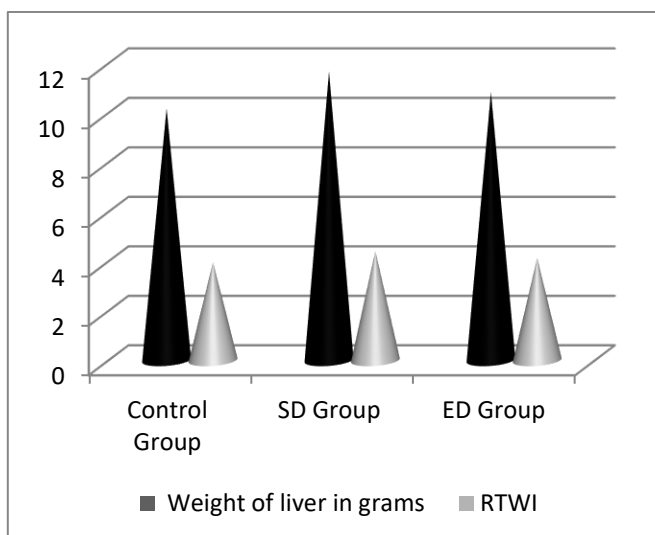


Figure.2: Weights of livers and RTWI of rats of control group, SD and ED group.

3. Relative tissue weight index:

The relative tissue weight index of each group was calculated and it was 3.97 ± 0.34 , 4.42 ± 0.56 and

4.14 ± 0.47 for control, SD and ED groups respectively. The difference among these groups came out to be statistically significant as shown after application of one-way ANOVA with p-value 0.036 (Fig.2) RTWI of SD group is higher as compared to control (p value 0.029) whereas of ED group, it was insignificantly higher than control group (p-value 0.574) that was evident by applying Tukey's test.

DISCUSSION

Current study revealed that average body weight of rats of SD group, receiving caffeinated soft drink (Coca Cola) and that of ED group, receiving caffeinated energy drink (Red Bull) was more than that of control group, but the difference was statically insignificant. These findings are consistent with that of Ebuehi et al, and Celec et al, who reported insignificant gain in body weight of experimental animals compared to control with energy drink and cola drink ingestion respectively.^{24,25} Tamura also noted insignificant raise in body weight of experimental animals when given coca cola.²⁶ According to Bukhar et al, low dose of energy drink caused insignificant gain in weight of rats, whereas the same energy drink in high dose significantly reduced the weight of rats.²⁷ Ugwuja with his associates also reported no significant gain in weight of experimental animals fed on energy drinks.²⁸ On the other hand, Bray²⁹ as well as Belpoggi showed increased in body weight of animals with soft drinks consumption.²⁰ Caffeine intake has been linked to less food intake and gastrointestinal upsets.^{30,31} Both Coca Cola and Red Bull, have caffeine, which can be held responsible for the insignificant gain in body weight of rats in our study. Augmented physical & mental activity with SD and ED consumption may also be held responsible for insignificant gain in weight.²⁷ Taurine has also shown to reduce animal weight in an experimental study,³² presence of taurine in ED may be held responsible for the insignificant weight gain besides presence of large amount of sugar.

Another finding in our study, is that there is no significant difference in average weight of livers of control group, SD and ED. Similarly, Ebuhei et al showed insignificant difference in weights of organs of rabbits when given energy drinks or caffeine.²⁴ Celec et al were also unable to show significant raise in weight of organs with consumption of cola drinks.²⁵ On the other hand, weight of rat liver was reported to be increased with high dose of energy drink consumption.²⁷ Changes in liver weight can occur in absence of morphological change in the liver. Normalization of liver weight to body weight (RTWI) helps to eliminate the variations due to differences in body weight.

The swelling of the cells leads to increase weight of the organ,³³ whereas necrosis of the cells tends to decrease the weight of the organ.³⁴ Possible explanation for insignificant rise in weight of organ may be that both cellular processes were simultaneously taking place in hepatocytes resulting in insignificant weight gain of the organ.

CONCLUSION:

Caffeinated soft drinks and energy drinks caused an increase in body weight and liver weight but not statistically significant increase. However, RTWI was also increased which means the consumption of these soft drinks and energy drinks, at a rate of 3 cans per day (11ml/kg) for just 8 weeks, may have started showing their injurious effects. This may be due to the caffeine content of these drinks, so the drinks should be used with caution when they are consumed in larger quantities over prolonged period of time especially by youngsters.

ACKNOWLEDGEMENTS

The animal house of Punjab Postgraduate Medical Institute, Lahore, was used by the kind permission of Prof Fauzia Farzana.

Photography of histology slides was allowed at University of Health Sciences, Lahore by Head of Anatomy, UHS, Lahore.

ETHICAL APPROVAL

The study was approved from Ethical Review Committee of Federal Postgraduate Medical Institute /Shaikh Zayed Hospital, Lahore, Pakistan. IRB No. 1207 Dated 23 November 2012.

REFERENCES

- Nseir W, Nassar F, Assy N. Soft Drinks consumption and nonalcoholic fatty liver disease. *World J Gastroenterol.* 2010;16(21):2579-2588.
- Grasser EK, Miles-Chan JL, Charrière N, Loonam CR, Dulloo AG, Montani JP. *Adv Nutr* 2016;7:950–960.
- The Secret Coca-Cola Formula Revealed! URL <http://communitytable.com/18045/cocacola-recipe-ftp/>
- Ferreira SE, Quadros IMH, Trindade AA, 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):841-847.
- Higgins JP, Babu KM. Caffeine Reduces Myocardial Blood Flow During Exercise. *The American Journal of Medicine.* 2013;126:730.e1-730.e8.
- Kerrigan S, Lindsey T. Fatal caffeine overdose: two case reports. *Forensic Sci. Int.* 2005 Oct 04;153(1):67-69.
- Glade MJ. Caffeine-Not just a stimulant. *Nutrition.* 2010; 26(10):932-938.
- Daniels JW, Mole PA, Shaffrath JD. Effects of caffeine on blood pressure, heart rate, and forearm blood flow during dynamic leg exercise. *J Appl Physiol.* 1998;85:154-159.
- Kugelman A, Durand M. A comprehensive approach to the prevention of bronchopulmonary dysplasia. *Pediatr. Pulmonol.* 2011;46(12):1153-1165.
- Ibrahim NK, Iftikhar R. Energy drinks: Getting wings but at what health cost?. *Pak J Med Sci.* 2014;30(6):1415-1419.
- Chen LW, Wu Y, Neelakantan N, Chong MF, Pan A, van Dam RM. Maternal caffeine intake during pregnancy is associated with risk of low birth weight: a systematic review and dose-response meta-analysis. *BMC Med.* 2014 Sep 19;12:174.
- Chen LW, Wu Y, Neelakantan N, Chong MF, Pan A, van-Dam RM. Maternal caffeine intake during pregnancy and risk of pregnancy loss: a categorical and dose-response meta-analysis of prospective studies. *Public Health Nutr.* 2016 May;19(7):1233-1244.
- Reissig CJ, Strain EC, Griffiths RR. Caffeinated energy drinks – A growing problem. *Drug Alcohol Depend* 2009;99:1-10.
- Luca AD, Pierno S, Camerino DC. Taurine: the appeal of a safe amino acid for skeletal muscle disorders. *J Transl Med.* 2015;13:243.
- Moloney MA, Casey RG, O'Donnell DH, Fitzgerald P, Thompson C, Bouchier-Hayes DJ. Two weeks taurine supplementation reverses endothelial dysfunction in young male type 1 diabetics. *Diab Vasc Dis Res.* 2010;7:300–310.
- Schaffer S, Kim HW. Effects and Mechanisms of Taurine as a Therapeutic Agent. *Biomol Ther.* 2018;26(3):225-241.
- Rosa FT, Freitas EC, Deminice R, Jordão AA, Marchini JS. Oxidative stress and inflammation in obesity after taurine supplementation: a double-blind, placebo-controlled study. *Eur J Nutr.* 2014;53:823–830.
- González-Contreras J, Villalobos Gámez JL, Gómez-Sánchez AI, García Almeida JM, Enguix Armanda A, Rius Díaz F, et al. Cholestasis induced by total parenteral nutrition: effects of the addition of Taurine (Tauramin®) on hepatic function parameters; possible synergistic action of structured lipids (SMOFlipid®). *Nutr Hosp.* 2012; 27:1900–1907.

19. Jurgens H, Haass W, Castaneda TR, Schurmann A, Koebnick C, Dombrowski F, et al. Consuming Fructose-sweetened Beverages Increases Body Adiposity in Mice. *Obes Res.* 2005; 13(7):1146-56.
20. Belpoggi F, Soffritti M, Tibaldi EVA, Falcioni L, Bua L, Trabucco F. Results of Long-term Carcinogenicity Bioassays on Coca-Cola Administered to Sprague_Dawley_Rats. URL http://www.laleva.org/it/img/NYAS_Coca-Cola_Ramazzini.pdf.
21. Stepien M, Duarte-Salles T, Fedirko V, Trichopoulou A, Lagiou P, Bamia C, et al. Consumption of soft drinks and juices and risk of liver and biliary tract cancers in a European cohort. *Eur J Nutr.* 2016;55(1):7-20. doi:10.1007/s00394-014-0818-5.
22. Seifert SM, Schaechter JL, Hershorin ER, Lipshultz SE. Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics.* 2011; 127(3):511-528.
23. Vivekanadarajah A, Ni S, Waked A. Acute hepatitis in a women following excessive ingestion of an energy drink: a case report. *Journal of Medical Case Reports.* 2011; 5:227.
24. Ebuehi OA, Ajayl OE, Onyeulor AI, Aweimobor D. Effects of oral administration of energy drinks on blood chemistry, tissue histology and brain acetylcholine in rabbits. *Nig Q J Hosp Med.* 2011; 21(1):29-34.
25. Celec P, Palffy R, Gardlik R, Behuliak M, Hodosy J, Jani P, et al. Renal and metabolic effects of three months of decarbonated cola beverages in rats. *Exp Biol Med (Maywood).* 2010; 235(11):1321-1327.
26. Tamura T, Fujii A, Kusaba H. Deleterious effect of short-term exposure to coca-cola on rats. *The Journal of Toxicological Sciences.* 1979; 4:363-376.
27. Bukhar HM, ElSawy NA, Header EA. Biological Effect of High Energy Drink on Normal and Hyperglycemic Rats. *Pakistan Journal of Nutrition.* 2012; 11(4):301-309.
28. Ugwuja E. Biochemical Effects of Energy Drinks Alone or in Combination with Alcohol in Normal Albino Rats. *Advanced Pharmaceutical Bulletin.* 2014; 4(1): 69-74.
29. Bray GB. Fructose: should we worry?. *International Journal of Obesity.* 2008; 32:S127-S131.
30. Fredhlm BB. Gastrointestinal and metabolic effects of methylxanthines. *Prog Clin Biol Res.* 1984; 158:331-54.
31. Hadas-Halpren I, Hiller N, Guberman D. Emphysematous gastritis secondary to ingestion of large amounts of coca cola. *Am J Gastroenterol.* 1993; 88(1):127-129.
32. Gunja N, Brown JA. Energy drinks: Health risks and toxicity. *Med J Aust* 2012;196:46-49.
33. Nayak NC, Sathar SA, Mughal S, Dutttagupta S, Mathur M, Chopra P. The nature and significance of liver cell vacuolation following hepatocellular injury- an analysis based on observations on rats rendered tolerant to hepatotoxic damage. *Virchows Arch.* 1996; 428:353-365.
34. Sundberg JP, Bedigian H. Focal Hepatic Necrosis in Inbred Laboratory Mice JAX® NOTES. Winter. 1994; 456.

AUTHORS' CONTRIBUTION:

SM: Ensured sample selection and carried out the experiment. Collected the data of this study for analysis & obtained results & organized discussion of study

SN: Organized introduction and literature review of the study

KN: Wrote citation & references of this study

MS: Supervised preparing manuscript according to journal criteria