Original Article

Effects of Caffeinated Soft Drink on Gonadosomatic Index and Testicular Histology: An In-Vivo Experimental Study in a Wistar Albino Rat Model

Shoukat Ali Memon[1], Sajjad Ali Almani[1] and Abdul Hafeez Baloch[2]

ABSTRACT

Objective: To determine the effects of caffeinated carbonated soft drinks on the testicular indices, gonadosomatic index (GSI) and histology in an *in-vivo* Albino Wistar rat model.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Medicine, Faculty of Medicine and Allied Medical Sciences, Isra University Hyderabad from July 2014 to February 2015.

Materials and Methods: The present experimental case control study was conducted at the Department of Anatomy and Postgraduate laboratory of Isra University Hyderabad Sindh. A sample of 20 Wistar Albino rats was selected randomly according to inclusion criteria. Group A (n=10)- tagged as control rats and Group B (n=10)- Experimental rats. Testicular size, weight and histological examination were performed. SPSS 22.0 was used for data analysis at 95% confidence interval (P-value ≤ 0.05).

Results: Body weight was increased in the experimental group B rats (P=0.0001). Testicular size and weight were decreased in rats given caffeinated soft drinkers (P= 0.0006). Necrotic areas, Edematous tissues, pyknotic nuclei, hyalinization, luminal defects, reduced seminiferous cell thickness; diameter and tubular lumen were noted in experimental rats.

Conclusion: Caffeinated Soft Drink showed deleterious effects on body weight, testicular indices, gonadosomatic index and testicular histology as observed in present in-*vivo* Wistar Albino Rat model study.

Key Words: Caffeine, Soft Drinks, Gonads, Body mass

Citation of article: Memon SA, Almani SA, Baloch AF. Effects of Caffeinated Soft Drink on Gonadosomatic Index and Testicular Histology: An In-Vivo Experimental Study in a Wistar Albino Rat model. Med Forum 2017;28(2):71-75.

INTRODUCTION

Biochemically, the caffeine is a methyl xanthene compound which is a potent brain stimulant hence commonly used in the soft drinks. Caffeine is the World's widely consumed psychoactive agent used as drug combinations and in soft drink beverages.[1] Caffeine is related to the purine bases (adenine and guanine) of the Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA). Most common source of caffeine is the seed of Coffea plants. Caffeinated carbonated soft beverages are consumed for vigor, vitality and prevent drowsiness and to improve physical performance.

[1.] Department of Anatomy, Isra University, Hyderabad.
[2.] Department of Anatomy, King Faisal University, College of Medicine, Al-Ahsa, Kingdom of Saudi Arabia.

Correspondence: Dr. Shoukat Ali Memon, Assistant Professor of Medicine, Isra University, Hyderabad, Sindh.
Contact No: 0300-3032792
Email: Giggly786@gmail.com

Received: November 22, 2016; Accepted: December 27, 2016

Caffeinated carbonated soft drinks are very popular; for example 90% of North Americans consume caffeine daily.[2,3] Caffeinated carbonated soft drinks increase body weight due to the sugar content. Obesity is a major health problem in the developed countries like America. Caffeinated carbonated soft drinks are linked to the Infertility and obesity.[4]

Exact recipe of Caffeinated carbonated soft drinks is one of the top most secrets of the industry. But the ingredients labelled on the tins of caffeinated carbonated soft drinks show containing caffeine, flavoring agents, colorants, odorants, phosphoric acid and bicarbonate.[5] Many countries have legally banned the use of caffeinated carbonated soft drinks in the schools and university students such as the Philadelphia, Los-Angeles and Miami. California bas legally banned the use of soft drinks by passing a Government Resolution in 2016 as they are bad for health.[6] Caffeine is a methyl Xanthine, an inhibitor of Phosphodiesterase (PDE) enzyme. Caffeine modulates the cellular functions by increasing the intracellular c-AMP levels, this results in the nervous system alert, increased appetite and physical activity.[7-9] Caffeine increases the basal metabolic rate and increases body temperature, this might interfere with testicular

functions, spermatogenesis, decreased sperm counts and infertility. The Gonadosomatic index, (GSI) is an index of percentage of gonad weight in relation to the body mass. GSI is an index of identifying the fertility status of an animal.GSI is calculated as [Gonad Weight / Total Tissue Weight] x 100.[10,11] A recent study reported the GSI is a good indicator of gonadal development and fertility in fish model during the spawning periods.[11,12] In this context, the effects of Caffeinated carbonated soft drinks needs further research as it may be causing serious fertility effects in human beings which is yet to be evaluated. The present study was designed to study the effects of caffeinated carbonated soft drinks on the testicular indices, Gonadosomatic index (GSI) and histology in an *in-vivo* Albino Wistar rat model. The present is the first study which determined the effects of caffeinated carbonated soft drinks on the testes. Testicular weight, body weight and testicular histology were the studied to determine the health hazardous effects of soft drinks. The present study hypothesized the caffeinated carbonated soft drinks have no adverse effects on the testes of albino Wistar rats.

MATERIALS AND METHODS

The present experimental case control study was conducted at the Department of Anatomy and Postgraduate laboratory of Isra University Hyderabad Sindh. The animals were housed at the Animal House, Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. The study covered a period of six months for experimentation and write up. A sample of 20 Wistar Albino rats were selected randomly according to inclusion criteria of body weight 200 grams and age 8 – 12 weeks. Animals were divided into 2 groups; Group A (n=10)- tagged as control rats and Group B (n=10)- Experimental rats which were used for the research purpose and were drink on caffeinated carbonated soft drinks along with normal chow diet. Study protocol was approved by the ethical review committee of the institute and the animal research ethics committee. Animals were kept in according to the NIH (National Institutes of Health) Guidelines for the Care and Use of Laboratory Animals. Plastic cages were used for the housing of rats. The animal house is well equipped. The cages are equipped with stainless steel feeders. Plastic drinkers with nozzles are available for the drinking purpose. Access to the water and standard chow diet was free and available 24 hours before and after experimental period. Saw dust was used as feeding and changed daily. Experimental group B rats were given caffeinated carbonated soft drinks in addition to the standard chow diet. Hygiene and ventilation of cages was strictly maintained. Temperature was maintained at the 26^0C. 12/12 hours dark and light cycles were maintained for the rats. Body weight was recorded on an electronic weighing machine before experiment. The caffeinated carbonated soft drinks were given for 30 days. At the end of experiment period, the body weight was performed again and noted. Animals were euthanized by cervical dislocation after Ketamine and Xylazine anesthesia. Body cavity was open by dissection; testes were retrieved by fine dissection. Gross examination of testes including size and weight were noted. Tissue was processed in formalin. 3-5µ thick tissue sections were prepared and stained by Hematoxylin and Eosin staining for the histological study. Histological slides were prepared from the testes of control and experimental rats both. Gonadosomatic index was calculated as using formula; Gonadosomatic index (%) = [Gonad Weight / Total Tissue Weight] x 100.[12] The data was analyzed on SPSS version 22.0 (IBM, incorporation, USA) for windows. Continuous variables comparisons were analyzed by Student t-test and results were presented as mean ± SD. While the categorical data was handled by Chi- square test and results were presented as frequency and %. Data was presented in tabulated form. 95% confidence interval was the criterion for the data analysis (P-value ≤ 0.05) as statistically significant.

RESULTS

Body weight and testicular parameters are shown in the table 1. The initial weight of the body in grams of Albino rats in groups A and B were as 218.80± 12.5 and 221.20 ± 9.7 respectively (P=0.057). End experiment body of Albino rats was noted as 226.40 ± 9.56 and 236.90 ± 16.29 in groups A and B respectively (P = 0.001). Testicular size was decreased in rats given caffeinated soft drinks. Testicular size was noted as 1.852±0.041 versus 1.749±0.067 in group A and B respectively (P= 0.0006).

Table No.1: Body weight and testicular parameters of experimental Albino rats (grams)

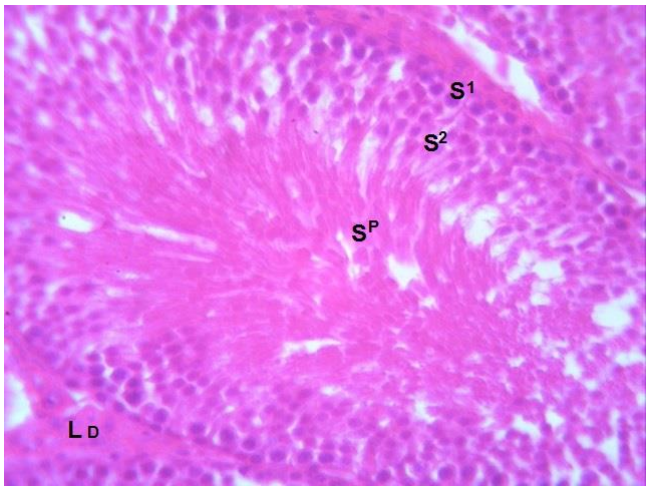
Groups	Group A	Group B	P-value
Body weight (baseline)	218.8±12.5	221.2±9.7	0.057†
Body weight (post-experiment)	226.4±19.1	236.9±25.4	0.001
Testicular size (cm)	1.852±0.041	1.749±0.067	0.006
Testicular weight (g)	1.068±0.027	0.860 ±0.063	0.0001
Tubular epithelial cell layers	5.20±0.63	2.90±0.57	0.0001
Seminiferous tubule diameter (µm)	259.8±4.01	257.2±3.9	0.0001

†- P value Non-significant

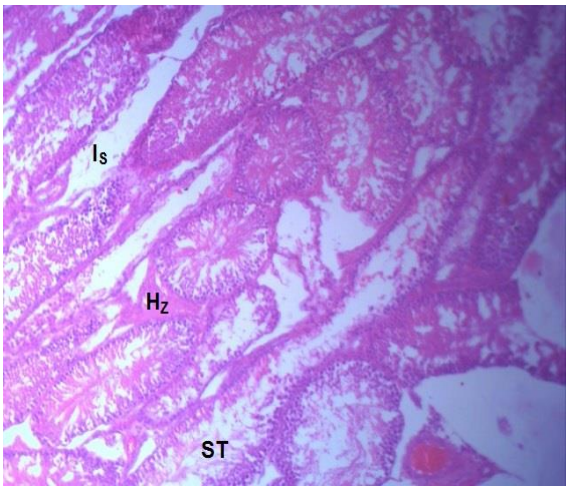
Table No.2: Gonadosomatic index (%) of experimental Albino rats

Groups	Mean	SD	P-value
Group A	0.47	0.02	0.001
Group B	0.37	0.04	

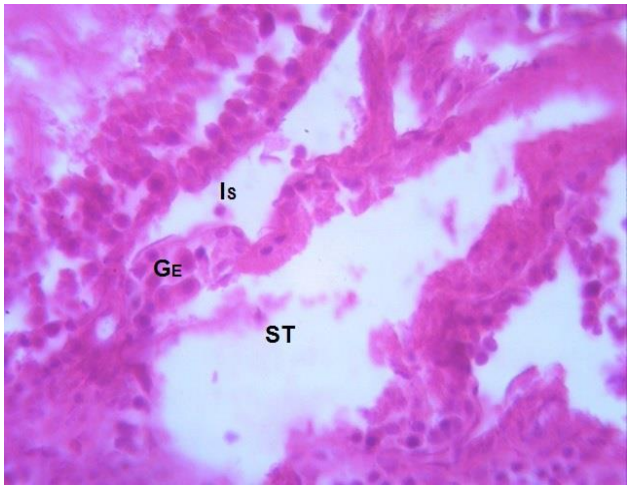
Testicular weight was found in gram in control group A and experimental groups B were 1.068±0.027 and 0.860 ±0.063 (p< 0.0001). The findings suggest a negative effect of caffeinated soft drinks on weight of testes. The GSI was decreased in rats that were given caffeinated soft drinks. GSI is mentioned in table-4. The GSI was decrease in experimental groups B (caffeinated soft drinks) as Compared to control group. GSI was found in control group A and experimental groups as 0.47 ± 0.02 and 0.37 ± 0.04% respectively. The GSI of experimental groups B showed a statistically highly significant decrease (P=0.0001). Histological findings of controls and experimental albino rats are shown in microphotographs 1 to 4. Testicular histology in experimental group B showed major changes in testicular histology as shown in microphotograph 2 to 4. Necrotic areas, Edematous tissues, pyknotic nuclei, hyalinization, luminal defects, reduced seminiferous cell thickness; diameter and tubular lumen were noted in rats given free quantities of mixture of caffeinated soft drinks.



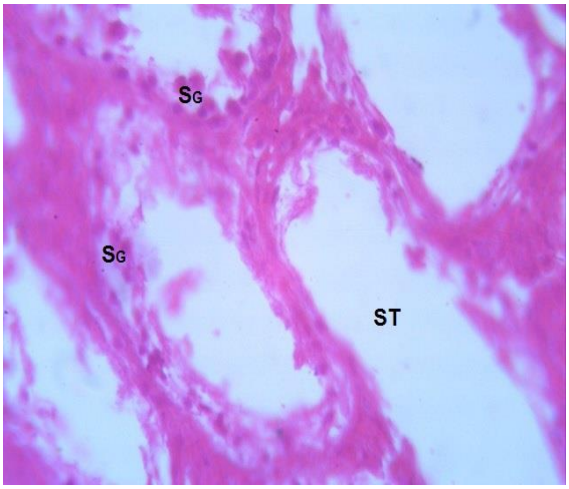
Microphotograph 1: Photomicrograph of the cross section of testis in control group A showing many normal Dark staining nucleus of spermatogonia (S1) present on the basement membrane. Spermatid (S2) is seen in adluminal surface. Luminal are filled with sperms (SP). Leydig cells are seen in the connective tissue. Magnification x 400. H&E



Microphotograph 2: Photomicrograph of the seminiferous tubules (ST) showing changes with the formation of edematous tissues in the interstitial (Is) connective tissue, necrotic areas, hyalinization (Hz) observed in the experimental group B (non-caffeinated soft drinks) x 100. H&E



Microphotograph 3: Cross section of the seminiferous tubules (ST) showing decrease layers of germinal epithelium (GE) indicating that there may be arrest of spermatogonia, decrease size of the basement membrane and decrease formation of sperms. Edema is observed in the interstitial connective tissue (Is). Magnification x 400. H&E (Experimental Group B)



Microphotograph 4: Cross section of seminiferous tubule (ST) shows spermatogonia (SG) which may be arrested and caused reduced germinal layer. Edematous and narrowing of lumen were observed in the experimental group 'B' x 400. H&E

DISCUSSION

The present is the first original research study conducted to determine the harmful effects of caffeinated carbonated soft drinks on the body weight, testicular indices, Gonadosomatic index and testicular histological changes in an *in-vivo* animal rat model. To the best of knowledge and a search of Pubmed, Medlip and Pakmedinet, the present is the first research being reported from Isra University Hyderabad, Sindh, Pakistan. The researcher claim it appears to be the first research study of its kind reported from Pakistan. Body weight, testicular weight, testicular indices, GSI and testicular histological examination showed adverse effects in the Experimental group B rats compared to the controls. This proves the hypothesis that the caffeinated carbonated soft drinks adversely affect the testicular structure and functions. Testicular weight, shrunken size and impaired spermatogenesis (Microphotographs 2-4) point towards deleterious effects of soft drinks *in-vivo*. The food intake was increased and increased body weight point towards the tendency of obesity induced by soft drinks. This proves the fact that the caffeinated carbonated soft drinks are health hazardous and may contribute to the obesity and infertility when consumed over a long time period. Soft drinks are proved to act as an appetizer. The observation points towards possibility of increased risk of obesity, infertility and cardiac problems in human beings too. Shrunken testicular size of rats fed on caffeinated carbonated soft drinks proves tendency of testicular toxicity. The rats given combined caffeinated carbonated soft drinks showed significant decrease in testicular size ($p<0.05$). Hence it may be postulated that the caffeinated carbonated soft drinks increase the risk of problems like infertility, testicular dystrophy, impaired spermatogenesis, etc. Similarly, testicular weight as shown in our study was reduced up to highly significant value ($p=0.0001$) in rats that were given ($p=0.0001$). The findings suggested a deleterious effect of caffeinated carbonated soft drinks against testes. These findings are in keeping with previous studies which reported deleterious effects of caffeinated carbonated soft drinks on the liver[13], kidneys[14], brain[15], cerebellum[16], lateral and medial geniculate body.[17] A dynamic bone disease in rats with chronic kidney disease, fructose induce metabolic syndrome and glomerular hypertension, carcinogenic effects of aspartame, semen quality of a child in pregnant women using soft drink (one of its ingredient) during pregnancy were reported.[18] The GSI is an indicator of sexual maturity and was found decreased in rats drunk the caffeinated carbonated soft drinks (table 2) ($p=0.0001$). Adebiyi[19] demonstrated that the Gonadosomatic index is the indicators of gonadal development in the fish and production of the eggs. The findings of present study are in agreement with a previous study by Ebbeling[20] has reported the effects of cola drinks on rat body weight. Body weight gain was observed similar to present study and risk of obesity was concluded. Adjene[21] reported on the deleterious effects of soft energy drinks on body and brain tissue. Body weight was increased in rat group's drunken caffeinated carbonated soft drinks and adverse effects were also reported on the brain tissue. The findings of above study support the present research. Eluwahad[22] reported shrinkage and degeneration of Cerebellar cortical Purkinje cells in experimental rats. Cerebellar ortical cells showed hypertrophic dendrites, increased number of regenerating molecular and granular cells, degeneration and increase in Purkinje cells and white matter spongiosis. These findings indirectly support that the caffeinated carbonated soft drinks are toxic for various tissue organs of body.[23-25] Keeping in view, the findings of present study and review of available medical research literature, it is suggested the caffeinated carbonated soft drinks adversely affect the various tissue organs of organisms. In present study, the harmful effects on the testicular structure and function are evident sufficient to report and warnings may be issued on the related health problems of obesity, infertility and obesity related metabolic syndrome. The use of caffeinated carbonated soft drinks may be restricted and law implications are suggested like countries as in California. The present study has some limitations- first; the present study is an experimental animal model study, second; the sample size was very small. Hence the findings cannot be generalized and further large scale studies are recommended.

CONCLUSION

The present study reports deleterious effects of Caffeinated Carbonated Soft Drink on body weight, testicular indices, Gonadosomatic index and testicular histology as observed in *in-vivo* Wistar Albino Rat model. Testicular necrosis, edema, pyknotic nuclei, hyalinization, luminal defects, reduced seminiferous cell thickness; diameter and tubular lumen were observed. These findings may possibly lead to poor quality of semen and infertility which needs both experimental and clinical research.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Voiculescu M, Segarceanu A, Negutu M, Ghita I, Fulga I, Coman OA. The effect of caffeine on cerebral asymmetry in rats. J Med Life 2015;8(4): 476–482.
2. Ghany AE, Nagib RM, El-Saiyed HM. Hypolipidemic Effect of Caffeine Beverages in Fatty Liver Injured Rats. J Appl Sci Res 2012; 8(3):1502-1509.

3. Gunja N, Brown AJ. Energy drinks: health risks and toxicity. Med J Australia 2012;196 (1):46-9.
4. Rath M. Energy drinks: What is all the hype? The dangers of energy drink consumption. J Am Acad Nurse Practitioners 2012; 24 (2): 70-6.
5. Dorostghoal M, Efani N, Nooraei MP. Maternal caffeine consumption has irreversible effects on reproductive parameters and fertility in male offspring rats. Clin Exp Repro Med 2012; 39(4): 144-52.
6. Dusman E, Berti AP, Soares LC, Vicentini VEP. Cytotoxicity and mutagenecity of cola and grape flavored soft drinks in bone marrow cells of rodents. Food Sci Techno Campinas 2013;33(1): 122-6.
7. Aghili HA, Hoseini SM, Yassaei S, Fatehmeybodi SA, Zaeim MHT, Moghadam MG. Effects of carbonated soft drink consumption on orthodontic tooth movements in rats. J Dent 2014;11(2): 123-30.
8. Jensen TK, Swan SH, Skakkebaek NE, Jorgensen RS. Caffeine intake and semen Quality in a population of 2,554 young Danish Men. Am J Epidemiol 2010;23:45-8.
9. Ramlau-Hansen CH, Thulstrup AM, Bonde JP, OlsenJ, Bech BH. Semen quality according to prenatal coffee and present caffeine exposure: two decades of follow up of a pregnancy cohort. Human Repro 2008;23(12):2799-2805.
10. Botezelli JD, Dalia RA, Reis IM, Barbieri RA, Rezende TM, Pelarigo JG, et al. Chronic consumption of fructose rich soft drinks alters tissue lipids of rats. Diaetol Metabol Syndr 2010; 2:43.
11. Tothova L, Hodosy J Mettenburg K, Fabryova H, Wagnerova A, Babickova J, et al. No harmful effects of different coca cola beverages after 6 months of intake on rat testes. Food Chem Toxicol 2013;62:343-8.
12. Amtyaz, M. Khan A, Khan MZ, Hashmi MUA. studies on Gonadosomatic Index &Stages of Gonadal Development of Striped piggy fish, Pomadasys stridens (Forsskal, 1775) (Family; Pomadasyidae) of Karachi Coast, Pakistan. J Entomol Zool Stud 2013;1 (5) 28-31.
13. Rath M. Energy drinks: What is all the hype? The dangers of energy drink consumption. J Am Acad Nurse Practitioners 2012; 24 (2): 70-6.
14. Khayyat L, Sorour J, Al Rawi M, Essawy A. Histological, ultrastructural and physiological studies on the effect of different kinds of energy drinks on the liver of Wistar Albino Rats. J Am Sci 2012; 8 (8):688-97.
15. Akande IS, Banjoko OA. Assessments of biochemical effect of Power Horse energy drink on hepatic, renal and histological functions in Sprague Dawley Rats. Ann Rev Res Biolog 2011;1(3): 45-56.
16. Adjene JO, Emojevwe V, Idiapho DE. Effects of long term consumption of energy drinks on the body and brain weights of adult WIstar rats. J Exp Clin Anat 2014;13(1):17-20.
17. Eluwa MA, Inyangmme II, Akpantah AO, Ekanem TB, Ekong MB, Asuquo OR, et al. A comparative study of the effect of diet and soda carbonated drinks on the histology of the cerebellum of adult female albino Wistar rats. Afr Health Sci 2013;13 (3):541-5.
18. Adjene JO, Emojevwe V, Igbigbi PS. Morphological effects of long term consumption of energy drinks on the intracranial visual relay centers of adult Wistar Rats. Ana J Afr 2014;3(1): 275-85.
19. Adebiyi FA. The Sex Ratio, Gonadosomatic Index, Stages of Gonadal Development and Fecundity of Sompat Grunt, Pomadasys jubelini (Cuvier 1830). Pakistan J Zool 2013;45(1):41-46.
20. Ebbeling CB, Feldman HA, Chomitz VR, Antonelli TA, Gortmaker SL, Osganian SK, et al. A randomized trial of sugar-sweetened beverages and adolescent body weight. The New Engl J Med 2012;367:1407–1416.
21. Adjene JO, Emojevwe V, Idiapho DE. Effects of long term consumption of energy drinks on the body and brain weights of adult WIstar rats. J Exp Clin Anat 2014;13(1):17-20.
22. Eluwa MA, Inyangmme II, Akpantah AO, Ekanem TB, Ekong MB, Asuquo OR, et al. A comparative study of the effect of diet and soda carbonated drinks on the histology of the cerebellum of adult female albino Wistar rats. Afr Health Sci 2013;13 (3):541-5.
23. De Ruyter JC, Olthof MR, Seidell JC, Katan MB. A trial of sugar-free or sugar-sweetened beverages and body weight in children. The New Engl J Med 2012;367: 1397–1406.
24. Sheludiakova A, Rooney K, Boakers RA. Metabolic and behavioral effects of sucrose and fructose/glucose drinks in the rat. Europ J Nutri 2012; 51: 445–54.
25. Sandner G, Angst MJ, Guiberteau T, Guignard B, Nehlig A. Effects of caffeine or RX821002 in rats with a neonatal ventral hippocampal lesion. Front Behav Neurosci 2014; 8 (15): 1-9.