Proceedings S.Z.M.C. Vol: 35(2): pp. 52-57, 2021. PSZMC-793-35-2-2021

# Flax Seed Oil Alleviates Caffeinated Energy Drink Induced Metamorphosis in Femur Weight and Relative Tissue Weight Index of Adult Male Albino Rat

[1]Afifa Waseem, [2]Muhammad Suhail, [2]Tayyaba Muzaffar, [2]Javed Iqbal, [3]Hadia Zulfiqar
[1]*Department of Anatomy, Rashid Latif Medical College, Lahore*
[2]*Department of Anatomy, Shaikh Zayed Medical Complex, Lahore*
[3]*Department of Anatomy, UCMD, University of Lahore*

## ABSTRACT

**Introduction:** Consumption of caffeinated energy drinks has been increased worldwide specially in younger population in order to increase mental and physical performance. Side effects on various systems of human body have been reported due to their usage. Flax seed oil has been well known since ancient times owing to its beneficial effects and has now become a major part in our functional food. **Aims & Objectives:** To evaluate the Protective effects of flax seed oil on caffeinated energy drink induced changes in adult male albino rat femur weight and relative tissue weight index. **Place and duration of study:** This study was accomplished in department of Anatomy, at Shaikh Zayed Postgraduate Medical Institute Lahore for 8 weeks. **Material & Methods:** 32 adult male albino rats average weight (250-300g) were randomly divided into four groups of 8 animals each. Group A (Control) received corn oil 5ml/kg body weight by gavage in addition to basal diet daily for 8 weeks. Group B (Experimental) received caffeinated energy drink (15ml/kg body weight) and corn oil 5ml/kg body weight. Group C (Experimental) received caffeinated energy drink (15ml/kg body weight) and 40% of flax seed oil (5ml/kg body weight), while group D (Experimental) received caffeinated energy drink (15ml/kg body weight) and 60% flax seed oil (5ml/kg body weight) daily for 8 weeks respectively. The animals were weighed before and after experiment. After 8 weeks, they were dissected to remove both femur and each femur was weighed, further the right femurs were used for statistical purpose. **Results:** The mean difference in weight of femur was nonsignificant amongst experimental groups (p= 0.950). The relative tissue weight index (RTWI) was significant (p-value 0.001). **Conclusion:** Flax seed oil helped in restoring altered femur weight and RTWI changes caused by caffeinated energy drink in adult male albino rats.

**Key words:** Flax seed oil, caffeinated energy drink, femur weight

## INTRODUCTION

Caffeinated energy drinks are a group of beverages which have occupied our markets worldwide in the last few decades. They are easily available in grocery stores and marketed as stimulant drinks used to replenish energy and to enhance endurance performance.[1,2] They mainly contain caffeine as a stimulant drug in addition to taurine, glucose, sucrose, glucuronolactone, vitamin B1, B2, B6, B12, artificial flavor and sparkling water.[3] The history of energy drinks dates back almost 130 years ago. During 20th century consumption of carbonated energy drinks with added caffeine increased worldwide.[4]

Caffeine ($C_8H_{10}N_4O_2$) is the world's most widely used psychoactive substance, belongs to methylxanthine class which is chemically related to adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). [5] It is naturally found in coffee beans, guarana seeds and cocoa beans. Many caffeine based energy boosting substances such as chocolate and its products, soft drinks, tea, coffee, ice-cream, pain, cold and flu medicines are commonly used in daily life.[6]

Caffeine acting as non-selective phosphodiesterases (PDE) inhibitor raises intracellular cyclic adenosine monophosphate cAMP, activates protein kinases-A, inhibits leukotrienes synthesis and reduces GABA production in tuberomamillary nucleus thus produces alertness.[7] Caffeine is metabolized in liver by P450 (CYP1A2) to active metabolites like

methylxanthine, theobromine and theophylline. It crosses blood brain barrier, placenta and can be found in breast milk.[8]

Long term use of caffeine not only leads to addiction and drug dependence but it can exacerbate risk of cardiovascular disease, type-2 diabetes mellitus, liver disease and bone resorption.[9] More than 300 mg a day can cause deleterious effects on human body and its intoxication can lead to tachycardia, hypokalemia, gastrointestinal disturbances, hallucinations, cerebral oedema, seizures, arrhythmias and even death.[10]

Caffeine works as a diuretic which may lead to urinary excretion of calcium phosphorus and magnesium even after several hours of its intake.[11] It also interferes with the absorption of vitamin D, which is essential for absorption of calcium from intestine. Various human studies have suggested that young adults and women who consume adequate calcium and moderate caffeine may have little or no deleterious effects. However, adults and older women who used to take more caffeine than compensated loss of calcium are at higher risk of osteoporosis.[12]

Evidences have suggested that dietary long chain polyunsaturated (PUFA), Omega-3 and Omega-6 fatty acids (ALA) can influence human health in diseased as well as in normal conditions. Additional studies have shown that diet rich in these ingredients have also positive effects on bone mass and their biomarkers.[13,14] Regarding to this aspect flax seed and its various products have occupied a major proportion of ancient medical history.[15] Flax seed or linseed (Linum Usitatissimum), has been cultivated for thousands of years by the ancient civilization of Ethiopia and Egypt for textile fiber and nutrition. Flax seeds come from the flowers of plants and can be pressed into oil and ground into flax seed meal for baking.[16]

Flax seed oil is rich in Omega-3, Omega-6 fatty acids (ALA), lignans, proteins, carbohydrates, Vitamin A, B1, B2, B6, C, E, folic acid and trace minerals like calcium, magnesium, phosphorus, potassium, copper and sodium .[17] The ALA content in flax seed oil is more than fish oil, so it can be used as an alternative to fish oil, where marine food is not available or danger of contamination of sea life. After regular use in our diet, Omega-3 fatty acids (ALA) not only reduces the risk of coronary disease, cholesterol, hypertension, metabolic syndrome and diabetes mellitus but also help in calcium and phosphorus absorption thus help in reducing inflammatory diseases like rheumatoid arthritis, osteoarthritis and osteoporosis.[18,19] Taking into consideration the above mentioned beneficial effects of flax seed oil on human body and due to extensive use of caffeinated energy drinks in youth, the current study was mapped out to mitigate the hazardous effects of caffeinated drinks on femur weight and relative tissue weight index (RTWI) of adult male albino rats.

## MATERIAL AND METHODS

32 adult, healthy male albino rats, age (3-6 months), average weight (250-300 g) were purchased from university of health science. They were divided into four groups, group A (control), group B, C and D (experimental groups), each group consisting of 8 rats. All the rats were kept at room temperature of 22± 25 ℃ .They were acclimatized for 7 days and had free access to food and water ad libitum. A 12 hours light/ dark cycle was maintained. After acclimatization, all the rats were divided randomly by lottery method into four groups. Before experiment all the rats were weighed and recorded. Furthermore the rats were marked with permanent markers for identification and placed in 4 different cages for 8 weeks.

**Group A:** Control group received corn oil 5ml/kg body weight daily for 8 weeks.

**Group B:** Experimental group received 15ml/kg of caffeinated energy drink and corn oil 5ml/kg body weight daily for 8 weeks

**Group C**: Experimental group received 15ml/ kg of caffeinated energy drink and 40% of flax seed oil (100ml oil formed by adding 40ml of flax seed oil and 60ml of corn oil) in a dose of 5ml/kg body weight daily for 8 weeks.

**Group D:** Experimental group received 15 ml/kg of caffeinated energy drink and 60% of flax seed oil (100 ml oil formed by adding 60 ml of flax seed oil and 40ml of corn oil) dose of 5ml/kg body weight daily for 8 weeks.

After 8 weeks of study period, all the rats were weighed individually, and then euthanized for dissection after 24 hrs of giving last doses of caffeinated energy drink and flax seed oil. A vertical midline incision was done on each thigh of the rat. The skin was reflected and muscles were removed to view femora. Both right and left femur were dissected out, cleaned and weighed individually. Furthermore, right femora were selected for statistical purposes.

**Statistical analysis:**

Data was entered and analyzed by using SPSS version 20.0. The quantitative variables like femur bone weight and relative tissue weight index (RTWI), were presented by using mean and

standard deviation (S.D). Comparison among group was made by using one way ANOVA. For post hoc analysis Tukey's was used where required. p-value≤ 0.005 was statistically significant.

## RESULTS

The mean weight of right femur in all groups was observed after experiment (Table-1).

One way ANOVA test revealed insignificant difference in mean femur weight among various with p = 0.950 (Table-2).

Post hoc Tukey test for multiple comparison of weight of femur in between control and experimental groups also showed nonsignificant difference among various groups (Table-3).

| Group | Weight of Bone | |
|---|---|---|
| | Mean ± SD | p-value |
| **A** | 1.245 ± 0.042 | 0.950+ |
| **B** | 1.231 ± 0.053 | |
| **C** | 1.236 ± 0.047 | |
| **D** | 1.240 ± 0.048 | |

**Table-1:** Weight of Femur (g) in control and experimental groups.

| | Sum of Squares | Df | Mean Square | F | p-value |
|---|---|---|---|---|---|
| Between Groups | 0.001 | 3 | 0.000 | 0.116 | 0.950+ |
| Within Groups | 0.063 | 28 | 0.002 | | |
| Total | 0.064 | 31 | | | |

**Table-2:** Comparison of weight of femur (g) of rats in control and experimental groups by using one way ANOVA

| Group | | Mean Difference | Std. Error | p-value |
|---|---|---|---|---|
| A | B | 0.013375 | 0.023807 | 0.943+ |
| | C | 0.009000 | 0.023807 | 0.981+ |
| | D | 0.004750 | 0.023807 | 0.997+ |
| B | C | -0.004375 | 0.023807 | 0.998+ |
| | D | -0.008625 | 0.023807 | 0.983+ |
| C | D | -0.004250 | 0.023807 | 0.998+ |

**Table-3:** Multiple comparison of weight of Femur (g) of rats in control and experimental groups by Tukey Test.

**2. Relative tissue weight index:**

The mean relative tissue weight index in all groups was observed after the experiment (Table-4).

A significant difference was found in mean RTWI among all groups with p = 0.001 (Table-5).

Group wise comparison revealed significant difference between experimental group B and control group A, while insignificant difference was found in remaining groups. (Table-6)

| Group | Relative Tissue Weight Index | |
|---|---|---|
| | Mean ± SD | p-value |
| **A** | 0.407 ± 0.008 | 0.001* |
| **B** | 0.388 ± 0.011 | |
| **C** | 0.397 ± 0.009 | |
| **D** | 0.399 ± 0.006 | |

Mean ± SD p-value
A 0.407 ± 0.008 0.001*
B 0.388 ± 0.011
C 0.397 ± 0.009
D 0.399 ± 0.006

**Table-4:** Relative Tissue weight Index of bone in control and experimental groups.

| | Sum of Squares | Df | Mean Square | F | p-value |
|---|---|---|---|---|---|
| Between Groups | 0.002 | 3 | 0.00051 | 6.899 | 0.001* |
| Within Groups | 0.002 | 28 | 0.00007 | | |
| Total | 0.004 | 31 | | | |

**Table-5:** Comparison of relative tissue weight index of right femur bone in control and experimental groups by using one way ANOVA.

| Group | | Mean Difference | Std. Error | p-value |
|---|---|---|---|---|
| A | B | 0.019500 | 0.004306 | 0.001* |
| | C | 0.010750 | 0.004306 | 0.082+ |
| | D | 0.008875 | 0.004306 | 0.191+ |
| | C | -0.008750 | 0.004306 | 0.201+ |
| | D | -0.010625 | 0.004306 | 0.087+ |
| C | D | -0.001875 | 0.004306 | 0.972+ |

*Significant difference(p value < 0.05)
+Insignificant difference (p value > 0.05)

**Table-6:** Multiple comparison of relative tissue weight index of right femur bone in control and experimental groups by using Tukey Test.

## DISCUSSION

Caffeinated energy drinks have raised special health concerns in the recent past years due to hazardous effects on various systems of human body like cardiovascular system, nervous system, gastrointestinal, reproductive system, muscles, joints, bones and mineral status of our body.[20] In the last few decades, public interest towards functional food has been developed due to potential health benefits of their ingredients. According to European commission, functional foods not only provide basic nutrition but also improve physical and mental health, thus decrease the risk of diseases.[21]

Flax seed and its various ingredients have potential health benefits due to their important ingredients like PUFA, MUFA, ALA, vitamin A, B, C, E, trace minerals such as calcium, phosphorus, magnesium, potassium and copper.[22]

In this study the mean right femur weight was decreased in experimental group B (received caffeinated energy drink) as compared to control

group A. While it was gradually increased in experimental group C & D (received caffeinated energy drink & 40% and 60% of flax seed oil) respectively as compared to experimental group B. (Table-1)

However, the main difference in femur weight was nosignificant among various groups. (Table-2 & 3). These findings are in relevance with the work of Shin et al, who also found reduction in length and weight of long bones exposed to high doses of caffeine for one month.[23] The increase in bone weight in group C and D as compared to group B was in both groups received 40% and 60% of flax seed oil along with caffeinated energy drink. The presence of PUFA in the form of ALA, omega-3 fatty acids and antioxidants in flax seed oil knocked out free radicals, helped to improve osteoporosis and bone weight in these groups.[24] This is in accordance with Hala, who suggested that flax seed oil PUFA and ALA helped to improve osteoporosis in estrogen deficient state.[14] It also coincides with the work of Weiss et al, who also found that high ratio of omega-3 and omega-6 PUFA helped in restoring bone mineral density and osteoporosis in both male and female.[25]

In the current research work the fluctuation in femur weight when compared with body weights of rats were calculated by RTWI. The mean difference in RTWI was statistically significant among various groups (p= 0.001 Table-4 & Table-5). It was relatively high in control group A as compared to experimental groups B, C and D. While, comparing groups it was gradually increased in group C and D (Caffeinated energy drink & 40% and 60% of flax seed oil) respectively. Multiple comparison of RTWI among groups revealed significant difference in between experimental group B and control group A (p = 0.001), however nonsignificant difference was observed among remaining groups B, C and D (Table-6).

The possible reason of decrease of RTWI in group B (caffeinated drink) as compared to control group A was due to decrease in the mean of femur weight and increase of mean body weight in this group. The probable reason of increase in mean body weight in this group was due to caffeinated energy drinks increased sugar level might impair insulin secretion and lipid storage in adipose tissue.[26] Similar findings were observed by Gheith, who also found significant increase in mean body weight in experimental group (caffeinated energy drink) as compared to control in adult male albino rats.[27]

The gradual increase in RTWI of group C (caffeinated energy drink & 40% flax seed oil) and group D (caffeinated energy drink & 60% flax seed oil) respectively was mainly due to consumption of flax seed oil in these groups which not only reduced osteoporosis and increased bone weight, but also helped in reducing the body weight of adult albino rats. This is in relevance to the work of El-Bahy et al, who also observed significant increase in mean bone weight and gradual reduction of mean body weight when treated with 5% and 10% of combination of flax seeds, pumpkin seeds and sesame seeds and thus RTWI was gradually increased in rats suffering from osteoporosis.[28] The probable reduction of osteoporosis and gain in bone weight had also been explained by Kelley, who postulated that PUFA and ALA in diet lower arachidonic acids and PGE-2 production and helped in bone repair by calcium absorption when given for 8 weeks in young adult malerats.[29] Also Maria et al, had emphasized that PUFA in the form of ALA along with EPA and DHA modify cytokines and inhibits COX-2, IL-1 and IL-6 and protein biosynthesis in osteoblastic cells thus, reduced inflammation and increased bone weight in male rats.[30]

## CONCLUSION

Flax seed oil helped in alleviating changes in femur weight and relative tissue weight index caused by caffeinated energy drink in adult male albino rats.

## REFERENCES

1. Al-Shaar L, Vercammen K, Lu C, Richardson S, Tamez M, Mattel J. Health effects and public health concerns of energy drink consumption in United States: A Mini-Review. Front Public Health. 2017; 5:225.
2. Terry- McElarth YM, O'Malley PM, Johnston LD. Energy drinks,soft drinks and substance use among United States secondary school students. Journal of Addiction Medicine. 2014; 8(1):6-13.
3. Visram S, Cheetham M, Ruby D, Crossley S, Lake A. Consumption of energy drinks by children and young people: a rapid review examining evidence of physical effects and consumer attitudes. 2016. BMJ Open;6:e010380 10.1136/bmjopen-2015-010380.
4. Robert Schinetsky. The complete history of energy drinks. 2018; 6(11). http://www.tigerfitness.com.
5. The caffeine from Wikipedia (free encyclopedia)2014; http://en.wikipedia.org/wiki/caffeine.
6. Whit Whitbread D. Top 10 foods and drinks high in caffeine. 22 Jan 2021. USDA Nutrition

data. Myfooddata.com/articles/high-caffeine-foods-and-drinks.php.
7. "Caffeine". The Pharmacogenetics and Pharmacogenomics Knowledge Base. Retrieved 25 October 2010. https://en.wikipedia.org/wiki/caffeine.
8. Van Dam RM, Hu FB, Willett WC. Coffee, Caffeine and Health. N Engl J Med. 2020; 383(4):369-378.
9. Petre A. What is caffeine, and is it Good Or Bad for Health? Health line Nutrition. 3 June2020. https://www.healthline.com/nutrition/what-is-caffeine.
10. De Sanctis V, Soliman N, Soliman AT, El Sedfy H, Di Maio S, El Kholy M, Fiscina B. Caffeinated energy drink consumption among adolescents and potential health consequences associated with their use: A significant public health hazard. Acta Biomed. 2017;88(2):222-31.
11. "Caffeine ''. Drug Bank. University of Alberta. Retrieved 2014. https://en.wikepedia.org/wiki/caffeine.
12. Nicholas H. What does caffeine do to your body? Caffeine: Benefits, risks and effects. 2017.https://www.medical newstoday.com/articles/285194#benefits.
13. Pessanha CR, etal. Brain development in male rats subjected to early weaning and treated with diet containing flour or flax seed oil after 21 days until 60 days. J Dev Orig Health Dis. 2015; 6:268-271.
14. Hala M. Hussein, MadihaM, Abdul –Kader, Seham S. Kasem. Flax seed down regulates bone resorption in knocked – out ovariectomized rats. 2010. I.J.A.R,3(2):1158-65
15. Goyal A, et al. Flax and flax seed oil: Ancient medicine & modern functional food. J FoodSciTeechnol. 2014; 51:1633-1653.
16. Skelton C. The origins and history of linseed and flax. May 2015. Info@flaxfarm.co.uk.
17. Iliades C. What are the health benefits of flax seed and flax seed oil? University Health News. 2020; Universityhealthnews.com/daily/nutrition/controversial-flax-seed-benefits.
18. Dr. Commane D. Flax seed consumption and bone metabolism in postmenopausal women. (FLAX). U.S National Library of Medicine NIH. 6 May 2018; ClinicalTrials.gov.Identifier: NCT03036722
19. Elbostany AN, Thabet HA and Ahmad HF. The effect of supplementation with flax seed and its extract on bone health.Nat.Sci.2013;11(5):71-80
20. Wiggers D, Asbridge M, Baskerville NB, Reid JL, Hammond D. Exposure to Caffeinated Energy Drink Marketing and Educational Messages among Youth and Young Adults in Canada. Int J Environ Res Public Health. 2019; 16(4)642.
21. Alshafe MM, Kaseem SS, Abdelkader MM, Hanafi ME. Flax seed as a Functional Food. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2020. ISSN:0975-8585. https://www.researchgate.net/publication/282282576.
22. Barham L. The health benefits of flax seed oil Dec 2020. Verywellhealth.com/flax seed-oil-health-benefits-how-to-use-caution417804#what-to-look-for
23. Shin J, Choi Y, Kim J, Yu AR, Shin JS, Choi YY, Roh J. High doses of caffeine reduce in vivo osteogenic activity in peripubertal rats. J Anat. 2015; 227(1):10-20.
24. Chen F, Wang Y, Wang H. etal. Flaxseed oil ameliorated high-fat-diet-induced bone loss in rats by promoting osteoblastic function in rat primary osteoblasts. Nutr. Metab (Lond) 2019; https://doi.org/10.1186/s12986-019-0393-0
25. Weiss LA, Barrett-Connor E, Muhlen DV. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study. Am JClinNutr. 2005; 81(4):934-938.
26. Bukhar HM, EISawy NA, Header EA. Biological effect of high energy drink on normal and hyperglycemic rats. Pakistan Journal of Nutrition. 2012; 11(4):301-309.
27. Gheith I. Clinical pathology of caffeinated and non-caffeinated energy drinks. Life ScienceJournal. 2017; 14(9):21-36.ISSN:1097-8135. http://www.lifesciencesite.com
28. EL-Bahy M, EL-Bendary S, Ashraf A,EL-Megeid A, Mahmoud E, Shoib YF, Hashem TM. Effect of flax seeds, pumpkin seeds and sesame seeds on calcium and phosphorus status of rats suffering from osteoporosis. 2011. http://www:nu.edu.sa/documents//61d29985-de9c- 486f_881a-f833322b34c
29. Kalleny N. Histological and morphometric studies on the effect of alpha-lipoic acid on postovariectomy osteoporosis induced in adult female albino rats. Egypt J Histol. 2011; 34:139-155.
30. Maria AD, Leticia PR, Costa DR, et al. Flax seed (Linumusitatissimum) flour contributes to bone health in adult male rats. Nutrition. 2018; (49):48-50. ISSN 08999-9007. 2017; 11.025

**The Authors:**
Dr. Afifa Waseem
Senior Demonstrator,
Department of Anatomy,
Rashid Latif Medical College, Lahore.

Prof. Muhammad Suhail
Head, Department of Anatomy,
Shaikh Zayed Medical Complex, Lahore.

Dr. Tayyaba Muzaffar
Assistant Professor,
Department of Anatomy,
Shaikh Zayed Medical Complex, Lahore.

Dr. Javed Iqbal,
Associate Professor,
Department of Anatomy,
Shaikh Zayed Medical Complex, Lahore.

Hadia Zulfiqar
Assistant Professor
Department of Anatomy,
UCMD University of Lahore

**Corresponding Author:**
Dr. Afifa Waseem
Senior Demonstrator,
Department of Anatomy,
Rashid Latif Medical College, Lahore.
E-mail: drafifawaseemrlmc@gmail.com