ORIGINAL ARTICLE

PROTECTIVE EFFECTS OF FLAX SEED OIL ON BODY WEIGHT CHANGES CAUSED BY CAFFEINATED ENERGY DRINK IN ADULT MALE ALBINO RATS

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ABSTRACT

Background: Flax seed oil has proven dynamic multisystemic effects since ancient times. Consumption of caffeinated energy drinks has also been increased among youth in order to increase mental and physical performance. Due to their widespread usage, hazardous effects on various systems of human body have been reported.

Objective: To evaluate the protective effects of flax seed oil on caffeinated energy drink induced changes in adult male albino rat body weight.

Methods: This study was conducted in FPGMI, Shaikh Zayed Hospital Lahore for 8 weeks. Thirty-two 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) were fed on 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.

Results: The mean body weight of rats before experiment was insignificant (p= 0.945). After experiment the mean body weight of experimental group B, C and D was increased as compared to control group A, but statistically it was insignificant (p = 0.319) however, percentage body weight gain was significant (p =0.003).

Conclusion: Flax seed oil alleviated altered body weight caused by caffeinated energy drink in adult male albino rats

Key words: Flax seed oil, caffeinated energy drink, Body weight, Male albino rats.

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INTRODUCTION

Caffeinated energy drinks are group of beverages used to replenish energy and to amplify endurance performance.[1] 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.[2]The history of energy drink usage dates back almost 130 years ago, while during 20th century consumption of carbonated drinks with added caffeine became a public health concern.[3]

Caffeine ($C_8H_{10}N_4O_2$) is the world's most widely used psycho active drug, a methylxanthine alkaloid and is chemically related to adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).[4] It is naturally found in coffee beans, gaurana seeds and cocoa beans. Many caffeine-based substances

such as tea, coffee, soft drinks, ice cream, pain, cold and flu medicines chocolate and its products are commonly used in daily life.[5]

Caffeine acting as non-selective phosphodiesterases inhibitor raises intracellular CAMP, activates protein kinases-A, inhbits leukutreins synthesis and reduces GABA production in tuberomamillary nucleus and produces alertness.[6-7] Its long term use is associated with inimical health concerns like coronary heart disease, type-2 diabetes mellitus , liver disease, infertility, osteoporosis , weight gain and metabolic syndrome.[8] More than 300 mg a day can have harmful effects on human body and its intoxication can lead to tachycardia, hypokalemia, gastrointestinal disturbances, hallucinations, cerebral oedema, seizures, arrhythmias and even death.[9] Caffeine is metabolized in liver by P450 (CYP1A2) to active methylxanthine, theobromine and theophylline. It crosses blood brain barrier, placenta and can be found in breast milk.[7-9]

The use of functional foods has also been increased globally not only due to their nutritional values but also safeguard against detrimental health problems.[10] Regarding this aspect flax seed and its various products have occupied a major proportion of ancient medical history.[11] Flax seeds are the seeds of plant called 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.[12]

This oil is rich in Polyunsaturated fatty acids (PUFA)Omega-3, Omega-6 fatty acid, ALA, eicosapentanoic acid (EPA), docosahexaenoic acid (DHA), Vitamin A, B1, B2, B6, C, E, calcium, magnesium, phosphorus, potassium and folate .[13] The ALA content in flax seed oil is more than fish oil, so it can be used as an alternative to fish oil where there is risk of contamination of marine life. Omega-3 and Omega-6 fatty acids not only reduces inflammatory conditions like rheumatoid arthritis, osteoarthritis but also provide protection against coronary artery disease, hypertention, hyperlipidemia, diabetes mellitus, chronic kidney disease (CKD), polycystic ovarian syndrome PCOS), metabolic syndrome and weight gain.[14,15]

METHODS

Thirty-two adult, healthy male albino rats, age (3-6 months), average weight (250-300 g) were purchased from university of health science Lahore. They were divided into four groups group A (control), group B, C and D (experimental groups), each group consisting of 8 rats. The weight of each rat was carefully recorded and then marked with permanent markers for identification and placed in 4 different cages for 8 weeks. A 12 hours light/ dark cycle was maintained. The animals had free access to food and water ad libitum.

Group A: Control group containing 8 animals and fed on corn oil 5ml/kg body weight by gavage daily for 8 weeks in addition to basal diet.

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

Data was entered and analyzed by using SPSS version 20.0. The quantitative variables for body weight, were presented by using mean + S.D and comparison among group was made by using one way ANOVA.

Tukey's test for post hoc analysis was used where required. Comparison among groups was made by using Chi-square test. P-value < 0.05 was considered significant.

RESULTS

Body weight of the rats (g): The mean body weight of rats before experiment was recorded. It was 257.1+ 11.7g, 259.0 + 7.5g, 256.1 + 9.9g and 257.5 + 8.6g for group A, B, C and D respectively (Table1, Fig. 1).

The mean body weight of rats after experiment was 305.6 + 12.8g, 317.5 + 13.6g, 311.5 + 11g and 311.0 + 12.1g for group A, B, C and D respectively (Table 1, Fig. 1).

Comparison of Body Weight of Rats Before and After Experiment: One way ANOVA test was applied to compare the body weight before and after experiment among groups. Insignificant difference was found in the mean body weight among animal of all groups before and after experiment ($p = 0.945$) and ($p = 0.319$) (Table 1) respectively. For multiple comparisons, post hoc Tukey test was used which also showed that the body weight of rats before and after experiment was insignificant in experimental groups B, C and D as compared to control group A. (Fig. 1&4)

Percentage body weight gain: The mean percentage body weight gain in all groups was observed. It was 18.9 + 1.7%, 22.6 + 2.8%, 21.7 + 0.9% and 20.8 + 1.4% in groups A, B, C and D respectively (Table.1). One way ANOVA test was applied to compare the percentage body weight gain among various groups which showed that the mean percentage body weight

gain was significant (p = 0.003) (Table 1)

Table 1: Body weight of rats (g) before and after experiment and percentage body weight gain among control and experimental groups.

Group	Percentage body weight gain after experiment			
	Before experiment Mean ± SD	After experiment Mean ± SD	Weight gain Mean ± SD	Percentage weight gain Mean ± SD
A	257.1 ± 11.7	305.6 ± 12.8	48.5 ± 3.9	18.9 ± 1.7
B	259.0 ± 7.5	317.5 ± 13.6	58.5 ± 7.9	22.6 ± 2.8
C	256.1 ± 9.9	311.5 ± 11.0	55.4 ± 2.1	21.7 ± 0.9
D	257.5 ± 8.6	311.0 ± 12.1	53.5 ± 4.6	20.8 ± 1.4
p-value	0.945+	0.319+	0.003*	

***** Significant difference (p < 0.05) + Insignificant difference (p > 0.05) - Constant Result

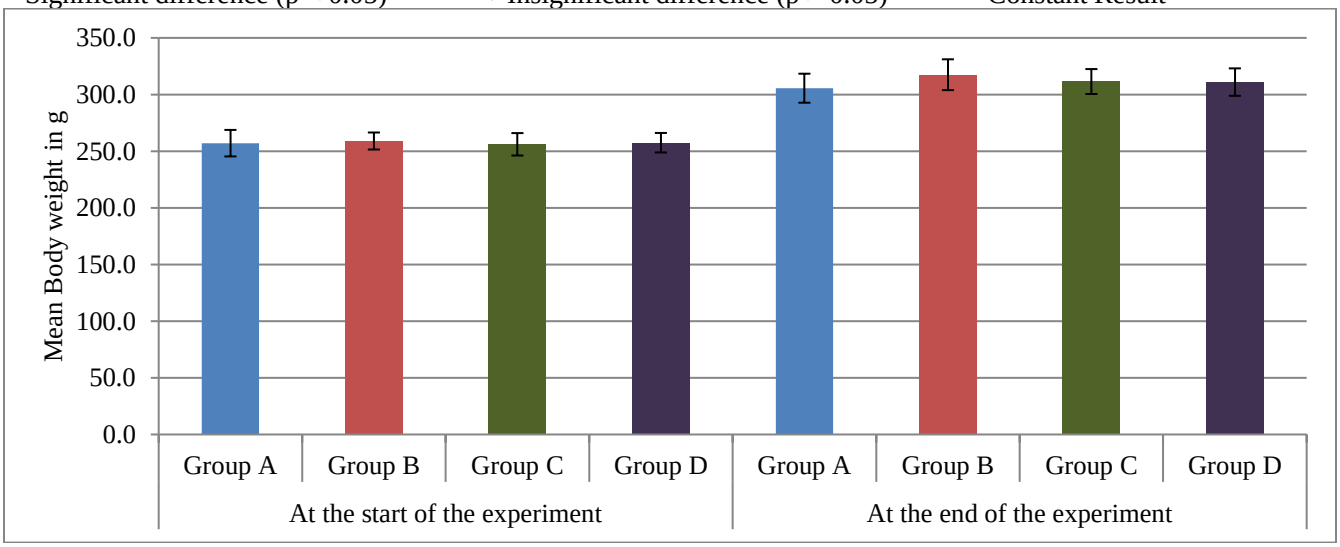


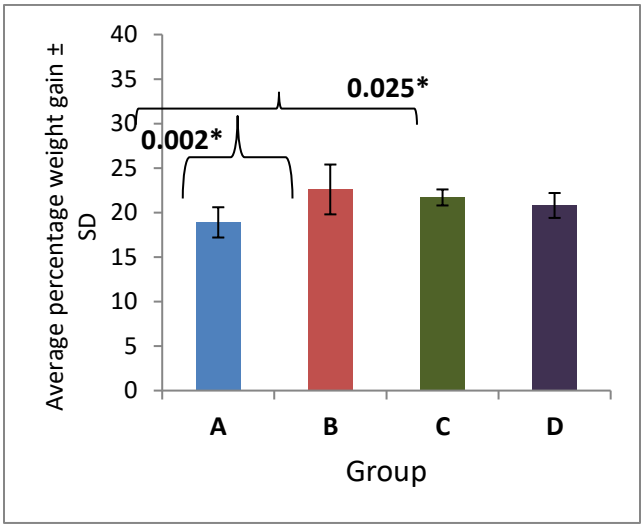
Figure.1: Comparison of body weight of animals among control and experimental groups.

For multiple comparisons, post hoc Tukey test was used which showed that the percentage body weight gain before and after experiment in between various groups was significant in experimental group B and C (p-value= 0.002 and 0.025) respectively as compared to control group A, but insignificant difference was found between control group A and experimental group D (table 2)

Table2: Multiple comparison of percentage body weight gain in control and experimental groups by Tukey Test

Group		Mean Difference	Std. Error	p-value
A	B	-3.6625	0.9071	0.002*
	C	-2.7500	0.9071	0.025*
	D	-1.8625	0.9071	0.193+
B	C	0.9125	0.9071	0.747+
	D	1.8000	0.9071	0.218+
C	D	0.8875	0.9071	0.763+

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



p = 0.003*

Figure 2: Comparison of percentage weight gain in animals among control and experimental groups.

DISCUSSION

Caffeinated energy drinks have emerged out in recent past years among youth with alarming health effects on major systems of human body like cardiovascular system, nervous system, gastrointestinal, reproductive system, bones and joints. They have gained special health concerns regarding hyperlipidemia, diabetes mellitus, body weight gain and metabolic syndrome due to caffeine and high sugar content.[16]

Flax seed and its various ingredients have got health benefits on various systems of our body due to their important ingredients like PUFA, MUFA, ALA in form of Omega-3, omega-6 fatty acids which not only provide protection against major diseases like coronary artery disease, hypertension, hyperlipidemia, diabetes mellitus, metabolic syndrome but also alleviate insulin resistance and altered body weight.[17]

In present study the mean body weight of all four groups was observed before and after experiment. Before experiment, mean body weight of animals in all groups was insignificant ($p = 0.945$) (table 1). After experiment, mean body weight of experimental group B (caffeinated energy drink) was increased as compared to control group A, however mean body weight was also increased in group C and D (caffeinated drink with 40% and 60% flax seed oil) respectively as compared to control group A (table 1) but statistically, it was insignificant ($p = 0.319$) (table 1). Multiple comparison of body weight of rats before and after experiment was also insignificant (Fig. 1).

These results coincide with the finding of Bukhar et al, who also observed insignificant weight gain in experimental groups administered caffeinated energy drinks as compared to control .[18]Minji et al, also observed insignificant weight gain with high doses of caffeine on body weight of peripubertal rats.[19]

The caffeine content of energy drink might be held responsible for the insignificant weight gain in group B which caused gastrointestinal disturbance and decreased consumption of food.[20] Another possible explanation can be that caffeinated drinks enhanced physical and mental activity and resulted in insignificant weight gain.[20-21]

Percentage body weight gain in same control and experimental groups was calculated, which was significant ($p = 0.003$) (table 1). Multiple comparison of percentage weight gain of animals also revealed significant difference in mean body weight of experimental group B and C as compared to control group A with ($p = 0.002$ and $p = 0.025$) respectively, while it was insignificant in between control group A and experimental group D (Fig. 2). These findings are in relevance with the work of Gheith, who also found significant increase in percentage body weight in experimental group (caffeinated energy drink) as compared to control in adult male albino rats.[22] The possible reason for increase in body weight of group B (caffeinated energy drink) might be due to increased sugar level causing increase lipid storage in adipose tissue.[23]

On contrary Eduardo et al, observed insignificant weight gain in adult male rats after 120 days administration of caffeinated energy drink and explained the thermogenic effect of high doses of caffeine which along with gastrointestinal disturbance might be responsible for weight control.[24]

Similar results were proved in a 4-week study conducted at University of Illinois that caffeine reduces body weight gain by decreasing storage of lipids and production of triglycerides.[25]

However, weight gain in group C and D was less (caffeinated energy drink, 40% and 60% of flax seed oil) respectively as compared to group B (caffeinated energy drink). The reason for insignificant weight gain in group C and D as compared to group B was that flax seed oil helped in weight reduction in these groups.[26]

McCullough et al, reported that consumption of flax seed oil significantly increased plasma and adipose level of ALA and leptin protein which have strong correlation with adipose levels and inverse correlation with obesity.[27] It had also been reported that flax seed oil significantly decreased the secretion of neuropeptide (NYP) which is a potent appetite stimulator and helped in weight reduction.[27]

Furthermore Omega-3 fatty acids in the form of ALA in flax seed oil not only regulate lipid metabolism but also ameliorated visceral and peripheral fat accumulation by reduction in production of cholesterol , triglycerides, LDL and VLDL.[28]Also Wu et al ,Costa et al and Boueri et al, emphasized the role of omega-3 fatty acids, ALA, lignans, SDG and DHA in flax seed oil which helped in weight reduction by improving dyslipedemia, glycemic control, insulin resistance thus reducing risk of metabolic syndrome.[29,30,31]

CONCLUSION

Flax seed oil has protective effect in mitigating body weight changes caused by caffeinated energy drink in adult male albino rats.

ETHICAL APPROVAL

The study was approved from Institutional Review Board of Federal Postgraduate Medical Institute, Lahore, Pakistan, vide reference No. F-38/NHRC/admin/IRB/161, dated July 13, 2016.

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AUTHOR'S CONTRIBUTIONS

AW: Concept and Design,Manuscript writing
MS: Supervision, Critical Review
TM: Data analysis, Literature Review
JI: Literature Review
HZ: References Research
SM: Data analysis, Critical Review