

Comparative Study of the Effect of Lipo-6-Black and Caffeine on the Fertility of Male Albino Rats

Yasameen Shaker Mahmood¹, Mukhtar Khamis Haba¹

¹Scholar Researcher, Department of Biology /College of Science for Women /University of Baghdad, Iraq

Abstract

This study was conducted to find out the effect of different doses of Lipo-6-black fat burner and Caffeine on the physiology of parts of the reproductive system of white male rats. The experiment included administering the Lipo-6-black fat burner to male white rats orally at concentrations (16.82, 8.41 and 4.20). Mg / kg)for 3-6 weeks , and also giving Caffeine orally, which is considered one of the main components of the fat burner at concentrations (11.42, 5.71, and 2.85 mg / kg) for a period of 3-6 weeks for each concentration. The study recorded the presence of weight changes of testis in addition to physiological changes, as it included the results of the first experiment were a decrease in in the weights of the testis and the levels of hormones (Testosterone, LH, FSH) at the middle and end of the experiment. As for the results of the second experiment, , changes in the weight of the testis , and an increase in the hormone levels themselves were observed at The middle of the experiment, while at the end of the experiment, no effect on hormones was shown.

Keywords: lipo-6-black, caffeine, fertility, male albino rats

Introduction

One of the most popular categories of nutritional supplements is often referred to as “fat burners” ⁽¹⁾. Many plants have medicinal importance because they contain active substances that are used as medicinal substances in human and animal treatments, after studying and purifying them. While there are other plants that are toxic to humans and animals because they contain active toxic substances ⁽²⁾. There are numerous studies on the effect of plant extracts or compounds isolated from them on the fertility of mammals. Among these substances, it may have a negative effect on the process of spermatogenesis, and sterility may occur ⁽³⁾. Caffeine is one of the most widely consumed food ingredients in the world. Every day, millions of people around the world use it in drinks and other foods that contain caffeine, including coffee, tea and chocolate ⁽⁴⁾. Caffeine in its pure state is in the form of a white powder. The bitter taste, and its chemical formula is C₈H₁₀N₄O,

its scientific name is 1,3,7-trimethylxanthine ⁽⁵⁾.

Methodology

Prepare the doses :

The lipo-6-black fat burner one capsule weighs 294.5 mg for a person. As for caffeine, an organization has defined The US Food and Drug Administration (FDA), the daily consumed dose, which was estimated at 200 mg / day ⁽⁶⁾. The amount of a single dose is (0.5 ml) in two doses per day, noting that the average weight of the rat was 250 g. The doses were prepared using the following equation:

$$X / \text{rat weight} = \text{dose given} / 100$$

Experiment animals:

In this study, 80 adult white male rats (40 per experiment), aged (8-10) weeks, with an average weight of about (270-250 g), were used in this study, obtained from the Animal House of the Cancer Research Center. The animals were placed in plastic cages. And the temperature ranged between (22-25 ° C) and humidity between (40-60%).

Corresponding author:

Yasameen Shaker Mahmood

Email: yasameenshakermahmood@yahoo.com

Experimental groups: The animals were divided in each experiment into four groups (each group contains 10 animals). In the first experiment, the animals were given the Lipo-6-black fat burner with concentrations of (16.82, 8.41 and 4.20 mg / kg). In the second experiment, Caffeine was administered with concentrations of (11.42, 5.71 and 2.85 mg / kg) of aqueous solution of both orally.

Blood collection: Blood was collected in two stages (three weeks and six weeks) from the experimental animals by a heart punctur method. The blood was kept in anticoagulant tubes (EDTA tube), then it was placed in a cooled centrifuge at a rate of 3000 rpm / minute at a temperature of 25°C for 10 minutes, in order to obtain the serum, after isolating the serum in sterile tubes, tests were performed to identify the Levels of the following hormones (Testosterone, LH, FSH) were done at the Biotechnology Research Center / Al-Nahrain University.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) and T-test was used to significant compare between means in this study (7).

Results and Discussion

Changes in testicular weight:

The results in the (Exp 1)showed a significant increase(p <0.05) in testicular weights of animals treated with Lipo-6-black (after 3weeks) compared to the control group. At the end of the experiment (after 6 weeks), the results showed a significant decrease (p <0.05) in testicular weights compared to the control group. Table (1). At the six-week period a significant decrease (p <0.05) was observed in the weights of the testicles, as it was indicated (8) that the gonads are rapidly affected by the change in nutrition compared to the glandular parts The other. The reason for the decrease in testicular weights may be due to the decrease in body weight, and this is in agreement with the study (3). In the second experiment, the results showed a significant increase (p <0.05) in testicular weights of animals treated with caffeine during the two periods, as shown in Table (1). The reason for increasing the weight of the testicles may be an increase in the number of sperm. This is in agreement with the study (9) in which it was observed that the weight of the testicles and other organs increased compared to the control group after administration of caffeine.

Table (1): the effect of lipo 6 black (Exp 1) and caffeine (Exp 2) for period (three and six weeks) on the testicular weights of rats, and a comparison of changes between the first and second experiments.

Week	Group	n	Mean ± SE of Testes weight (gm)		T-test
			Exp. 1	Exp. 2	
W3	Control	3	1.241 ± 0.006 c	1.251 ± 0.007 c	0.0123 NS
	G1	3	1.326 ± 0.001 b	1.258 ± 0.004 bc	0.0159 *
	G2	3	1.316 ± 0.006 b	1.275 ± 0.005 ab	0.0155 *
	G3	3	1.341 ± 0.001 a	1.291 ± 0.004 a	0.0164 *
	LSD value		0.014 *	0.0176 *	---
W6	Control	3	1.458 ± 0.015 a	1.495 ± 0.007 c	0.0275 *
	G1	3	1.336 ± 0.001 b	1.520 ± 0.007 b	0.0526 *
	G2	3	1.303 ± 0.011 b	1.526 ± 0.001 b	0.0437 *
	G3	3	1.193 ± 0.011 c	1.561 ± 0.008 a	0.103 *
	LSD value		0.037 *	0.0229 *	---

The means that carries different letters within the same column differs significantly between them. n represents the number of animals. * (P≤0.05), NS: Non-Significant

The effect on the level of hormones in male rats:

Testosterone:

In the first experiment, the results showed a significant decrease ($p < 0.05$) in the testosterone concentration of animals. The reason for this is that damage occurs in the leydig cells, which leads to a decrease in the level of the hormone testosterone responsible for the divisions of the cells that make up sperm during the process of spermatogenesis. The decrease in the concentration of the hormone causes damage in these cells. And then damage the seminal tubules in general, so damage to the leydig cells and the decrease in their numbers have a direct effect on the level of the hormone testosterone ⁽¹⁰⁾.

In the second experiment, a significant increase ($p < 0.05$) was found in the testosterone concentration of

animals (three weeks), and it did not show a significant difference after six weeks, as shown in the table (2). A significant increase was observed ($p < 0.05$) as caffeine could have an indirect effect on testosterone through its effect on the hypothalamo-pituitary-gonadal system ⁽¹¹⁾. This is consistent with what was stated in a study ⁽¹²⁾ which noted the high production of the hormone testosterone, which showed the non-toxic effect of caffeine and explained its effect on the leydig cells depending on the dose. This also agrees with study ^(13,14) which showed that high testosterone is related to caffeine. But after six weeks, no significant difference was observed in testosterone concentration, and this is in agreement with a recent study ⁽¹⁵⁾ which noted no correlation between Caffeine and testosterone level in men.

Table (2): The effect of lipo 6 black (Exp 1) and caffeine (Exp 2) for period (three and six weeks) on the Testosterone concentration in rats, and a comparison of changes between the first and second experiments.

Week	Group	N	Mean ± SE of Testosterone (ng/ml)		T-test
			Exp. 1	Exp. 2	
W3	Control	3	3.31 ± 0.07 A	3.36 ± 0.05 c	0.219 NS
	G1	3	3.02 ± 0.14 B	3.70 ± 0.08 b	0.437 *
	G2	3	2.70 ± 0.02 C	4.01 ± 0.13 ab	0.530 *
	G3	3	2.58 ± 0.02 C	4.11 ± 0.10 a	0.549 *
	LSD value		0.268 *	0.320 *	---
W6	Control	3	4.11 ± 0.11 A	4.18 ± 0.10 a	0.205 NS
	G1	3	1.57 ± 0.27 B	4.18 ± 0.02 a	0.629 *
	G2	3	0.820 ± 0.15 C	4.31 ± 0.02 a	0.533 *
	G3	3	0.413 ± 0.03 C	4.37 ± 0.05 a	0.518 *
	LSD value		0.553 *	0.198 NS	---

The means that carries different letters within the same column differs significantly between them. n represents the number of animals. * (P≤0.05), NS: Non-Significant

Luteinizing hormone (LH):

In the first experiment, the results showed a significant decrease(p <0.05) in the LH concentration of animals during the two experimental periods compared to the control group. This may be due to a problem in the testicles or due to a problem in the hypothalamic-

pituitary-gonadal axis and thus a deficiency of GnRH, which leads to a decrease in the production of LH, which subsequently leads to a decrease in sperm formation ⁽¹⁶⁾.

In the second experiment, a significant increase (p <0.05) was found in the LH hormone concentration during the three-week period, and it did not show a significant difference in the six-week period as shown in the table³⁾. This is in agreement with a study ⁽¹¹⁾ which showed that caffeine did not affect the LH level of male rats.

Table (3): The effect of lipo 6 black (Exp 1) and caffeine (Exp 2) for period (three and six weeks) on the LH concentration in rats, and a comparison of changes between the first and second experiments

Week	Group	N	Mean ± SE of LH (mIU/ml)		T-test
			Exp. 1	Exp. 2	
W3	Control	3	2.76 ± 0.19 A	2.38 ± 0.04 B	0.307 NS
	G1	3	2.58 ± 0.18 Ab	2.63 ± 0.05 B	0.266 NS
	G2	3	2.28 ± 0.02 Bc	3.36 ± 0.21 A	0.521 *
	G3	3	2.01 ± 0.09 C	3.44 ± 0.13 A	0.568 *
	LSD value		0.461 *	0.4198 *	---
W6	Control	3	3.44 ± 0.40 A	3.45 ± 0.41 A	0.238 NS
	G1	3	1.23 ± 0.29 B	3.68 ± 0.40 A	0.694 *
	G2	3	0.473 ± 0.16 Bc	3.85 ± 0.01 A	0.736 *
	G3	3	0.193 ± 0.01 C	3.87 ± 0.01 A	0.697 *
	LSD value		0.852 *	0.668 NS	---

The means that carries different letters within the same column differs significantly between them. n represents the number of animals. * (P≤0.05), NS: Non-Significant

Follicle stimulating hormone (FSH):

In the first experiment, the results showed a significant decrease ($p < 0.05$) in the FSH concentration during the two experimental periods compared to the control group. Low levels of FSH are associated with either a dysfunction of the hypothalamus or the anterior pituitary gland (16). This may be due to the effect of the fat burner on these glands and thus the effect of the FSH hormone in the adult males treated with the fat burner.

In the second experiment, a significant increase ($p < 0.05$) was found in the FSH concentration of animals during the three-week period, and it did not show a significant difference in the six-week period, as shown in the table (4). This is in agreement with a study (9), in which an increase in the level of FSH was observed in mature male rabbits after exposure to Caffeine for four weeks. And study (14) which showed that Caffeine had no effect on the FSH hormone in males exposed to Caffeine.

Table (4): The effect of lipo 6 black (Exp 1) and caffeine (Exp 2) for period (three and six weeks) on the FSH concentration in rats, and a comparison of changes between the first and second experiments.

Week	Group	n	Mean ± SE of FSH (mIU/ml)		T-test
			Exp. 1	Exp. 2	
W3	Control	3	2.33 ± 0.02 A	2.31 ± 0.04 B	0.206 NS
	G1	3	2.53 ± 0.18 A	2.53 ± 0.07 B	0.149 NS
	G2	3	2.22 ± 0.01 A	3.24 ± 0.18 A	0.385 *
	G3	3	1.79 ± 0.06 B	3.30 ± 0.17 A	0.477 *
	LSD value		0.318 *	0.429 *	---
W6	Control	3	3.31 ± 0.40 C	3.34 ± 0.38 A	0.152 NS
	G1	3	1.09 ± 0.29 B	3.58 ± 0.06 A	0.746 *
	G2	3	0.350 ± 0.15 Bc	3.74 ± 0.02 A	0.692 *
	G3	3	0.126 ± 0.01 C	3.76 ± 0.02 A	0.603 *
	LSD value		0.846 *	0.642 NS	---

The means that carries different letters within the same column differs significantly between them. n represents the number of animals. * ($P \leq 0.05$), NS: Non-Significant

Conculution

As it was shown from the results of the two experiments, and after a comparison between them, it was found that the fat burner had a negative effect on the levels of the following hormones (Testosterone, LH, and FSH) and thus affected the function of the testis in addition to the effect on the weight of the organ. As for caffeine, it did not have a negative effect on the same hormones and organ. Therefore, the use of a fat burner on male rats led to harmful changes, unlike caffeine.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

References

1. Al-Tae MF, Abdul-Malek HW, Majeed LJ. Evaluation of toxic effects of two weight reduction pills. *J Biotechnol Res Cent.* 2012;6(2):23–8.
2. Jeukendrup AE, Randell R. Fat burners: Nutrition supplements that increase fat metabolism. *Obes Rev.* 2011;12(10):841–51.
3. Sakamoto J, Hashimoto K. *Toxicology* 9. 1986;201–2.
4. Heckman MA, Weil J, de Mejia EG. Caffeine (1, 3, 7-trimethylxanthine) in foods: A comprehensive review on consumption, functionality, safety, and regulatory matters. *J Food Sci.* 2010.
5. SAS. 2012. *Statistical Analysis System, User's Guide.* Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
6. Hotzel MJ, Markey CM, Walkden-Brown SW, Blackberry M, Martin G. Effects of nutrition on testicular morphology and endocrinology in rams. In: *Effects of nutrition on testicular morphology and endocrinology in rams.* Australian Society for Reproductive Biology; 1995. p. 115.
7. Owolabi Joshua O, Olatunji Sunday Y, Olanrewaju John A. Excessive Caffeine Intake Disrupts Testicular Architecture, Spermatogenesis and Hormonal Levels in Experimental Wistar Rats. 2017.
8. Eteng MU, Eyong EU, Akpanyung EO, Agiang MA, Aremu CY. Recent advances in caffeine and theobromine toxicities: A review. *Plant Foods Hum Nutr.* 1997.
9. Sc O, Eg E. Effect of Caffeine (Nescafé) on Serum Testosterone Levels in Male Wistar Rats. *Sch J Appl Med Sci.* 2018.
10. Svartberg J, Midtby M, Bønaa KH, Sundsfjord J, Joakimsen RM, Jorde R. The associations of age, lifestyle factors and chronic disease with testosterone in men: The Tromsø study. *Eur J Endocrinol.* 2003.
11. Ramlau-Hansen CH, Thulstrup AM, Bonde JP, Olsen J, Bech BH. Semen quality according to prenatal coffee and present caffeine exposure: two decades of follow-up of a pregnancy cohort. *Hum Reprod [Internet].* 2008 Sep 1;23(12):2799–805.
12. Lopez DS, Advani S, Qiu X, Tsilidis KK, Khera M, Kim J, et al. Caffeine intake is not associated with serum testosterone levels in adult men: cross-sectional findings from the NHANES 1999–2004 and 2011–2012. *Aging Male.* 2019;22(1):45–54.
13. Clavijo RI, Hsiao W. Update on male reproductive endocrinology. *Translational Andrology and Urology.* 2018.
14. Ogunwole E, Akindele OO, Oluwole OF, Salami SA, Raji Y. Effects of Oral Maternal Administration of Caffeine on Reproductive Functions of Male Offspring of Wistar Rats. *Niger J Physiol Sci.* 2015.
15. Orłowski M, Sarao MS. Physiology, Follicle Stimulating Hormone. *StatPearls.* 2019.
16. Ezzat AR, el-Gohary ZM. Hormonal and histological effects of chronic caffeine administration on the pituitary-gonadal and pituitary-adrenocortical axes in male rabbits. *Funct Dev Morphol.* 1994.