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The Histopathological Effects of Animal Tallow and Vegetable Oil on Male Reproductive System of Rats

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Abstract. The aim of this study was to examine the effect of beef tallow and sunflower oil on testis tissue in rats. Fifteen male albino rats at four weeks of age and weighing between 35 g to 38 g were used and divided into three groups (5 rats each). The control group was fed standard diet while, the experimental groups were fed either sunflower oil-enriched diet or beef tallow-enriched diet. After four weeks, histopathological analysis was performed. Testis sections were stained with Hematoxylin and Eosin to investigate the histopathological alterations. The histopathological investigation showed irregular shape of seminiferous tubules and there were some vacuoles within the germ cells. Also, degeneration of several seminiferous tubules was observed in beef tallow group compared with control and sunflower oil groups. In conclusion, addition of beef tallow to growing rats caused several histopathological alterations in testicular tissues. The continuous consumption of high dietary fat can potentially cause detectable alterations on rat testes that negatively influence on sperm function and leading to fertility problems.

Keywords: Animal tallow, vegetable oil, testis histopathology, male reproductive system

INTRODUCTION

There is a growing social concern about the male infertility caused by continuous consumption of high dietary lipid resulting in several health problems. It was found that the consumption of dietary fat is associated with several diseases such as reproductive, liver and cardiovascular diseases (1). There are a large number of studies in the literature were designed to investigate the effect of dietary fat on the human body. However, little is known about their effects on the male reproductive system. Therefore, this study was designed to determine the effects of two different type of fat on rat male reproductive system. It was observed that the type of fat affects male reproductive system through decreasing the sperm quantity and motility, thus reducing fertilization rate (2,3). Interestingly, the amount and the type of fat in the diet play an important role in metabolic disturbances such as glucose tolerance, hepatic steatosis and dyslipidaemia and insulin resistance development (4,5). Furthermore, monounsaturated, polyunsaturated or saturated fat may cause various effects depend on the degree of fat saturation (6). The experimental studies in mice showed less body weight in mice feeding a corn oil-enriched diet for two weeks than did mice feeding a beef tallow-enriched diet (7). *In vivo* and *in vitro* studies observed that oxidation of saturated fatty acids is slower than that of polyunsaturated fatty acids. These results suggest that fat accumulation in the body is greater in animals consumed a saturated fatty acids-enriched diet with than in those consumed polyunsaturated fatty acids-enriched diet (8). In this context, it has been demonstrated that excess weight leads to reduce male reproductive system and effects the fertility and decline the rate of pregnancy (9). In present study, we attempt to compare the effects of two different fat on rat male testicular tissues by feeding animals either a sunflower oil diet or a beef tallow diet for one month

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MATERIALS AND METHODS

Animals care

A total of fifteen albino male Wistar Kyoto rats of four weeks of age and weighing between 35 g to 38 g were obtained from animal center of Veterinary College / Basrah University, (Basrah, Iraq). Animals were housed in animal center at College of Pharmacy, University of Basrah and they were kept in a 12 h light–dark cycles and a temperature of 25 ± 2°C. The animals were approved by the Animal Research Ethical Committee of Basrah University.

Diet preparation and experimental design

The rats were randomly divided into three groups with five animals in each group. The control group was fed a purified diet AIN-76 diet as a control diet. AIN-76 diet was accepted as general nutrition for experimental rodents therefore, it was used in this study for control group. The control diet contained the following ingredients, in grams per kilogram: sucrose 500 gm, casein 200 gm, cornstarch 150 gm, cellulose 50 gm, corn oil 50, mineral mix 35mg, vitamin mix10 mg, DL-Methionine 3gm and choline bitartrate 2 gm (10). The experimental groups fed high fat diet (20% wt/wt fat) containing sunflower oil or the beef tallow. Sunflower oil and beef tallow diets were prepared by adding 200 gm per Kg of fat (sunflower oil or beef tallow) to the same ingredients of the control diet (7). Each diet was freshly made every day throughout the experimental time. Sunflower oil was purchased from a super market and the animal tallow was extracted using adipose tissues of animals obtained from local slaughter houses. All animal groups were free access to drinking water and they were meal-fed the diet for four weeks.

Analysis of body and organ weight

The body weights of animals were measured before the onset of the study and prior to the sacrifice of the rats. The data were tabulated and statistically analyzed. Testicular weights were measured on analytical balance for relative weight analysis, using the formula: organ weight (g)/animal weight (g) X 100.

Testis histopathological analysis

For histological analysis, the rats were anesthetized then the testes were carefully removed from control and experimental groups. All testes cleared of the adhering connective tissue and then the measurement testis weight was evaluated. All testes samples were fixed in 10% formalin then processed with different levels of alcohol followed by xylene and then paraffin embedding. Sections of thickness 5 µm were cut using a rotary microtome. All slides were stained by hematoxylin and eosin (H&E) (11). The stained slides were viewed and photographed using a light microscope (Olympus EH) at different magnifications and used for histological analysis.

STATISTICAL ANALYSIS

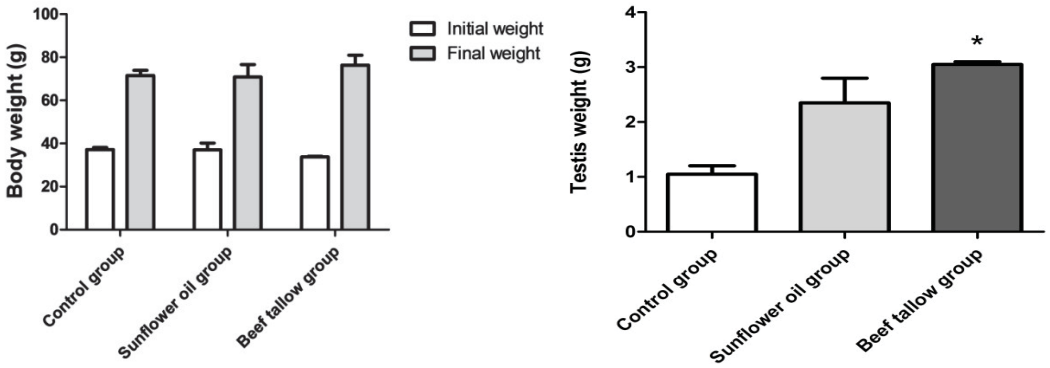
A one-way ANOVA was performed to compare data of animal body weights and relative weight of testis by using the software GraphPad Prism 5 for windows (San Diego, CA, USA). Post hoc comparisons were used by Bonferroni's multiple comparison tests (MCT). The results were presented as Mean ± Standard Error of Mean (SEM). The results were considered as significant when P-values < 0.05.

RESULTS

Animal and organ weight

Animals used in this work survived well during the experimental time. The mean body weight of the rats was measured before the onset of the study and at the end of the treatment period. There was an increase in the weight of

the rats but it was not statistically significant (Fig 1a). The mean testis weight was found to be significantly higher ($p < 0.05$) in beef tallow groups compared with control group and sunflower oil group (Fig. 1b).



(a) (b)

FIGURE 1. Body Weights and relaitve testis weight of rats. (a) Bar chart displaying the initial and final body weight of rat, No significance; one-way ANOVA, Bonferroni's post-test with error bars representing SEM. (b) Bar chart showing the relaitve weight of rat testis, *$p < 0.05$; one-way ANOVA, Bonferroni's post-test with error bars representing SEM.

Histological study

The histological evaluation of the testicular pathology of control and sunflower groups showed normal spermatogenic and Sertoli cells in the seminiferous tubules as shown in Fig. 2 and Fig. 3, respectively. However, H&E-stained sections of testicular tissue in beef tallow group showed several histopathological changes. Degeneration in the seminiferous tubules and depression of spermatogenesis were observed compare with the control and sunflower oil diet groups. Also, detachments of spermatogonia from the basal membrane and the presence of vacuoles in the epithelium of seminiferous tubule were shown (Fig. 4).

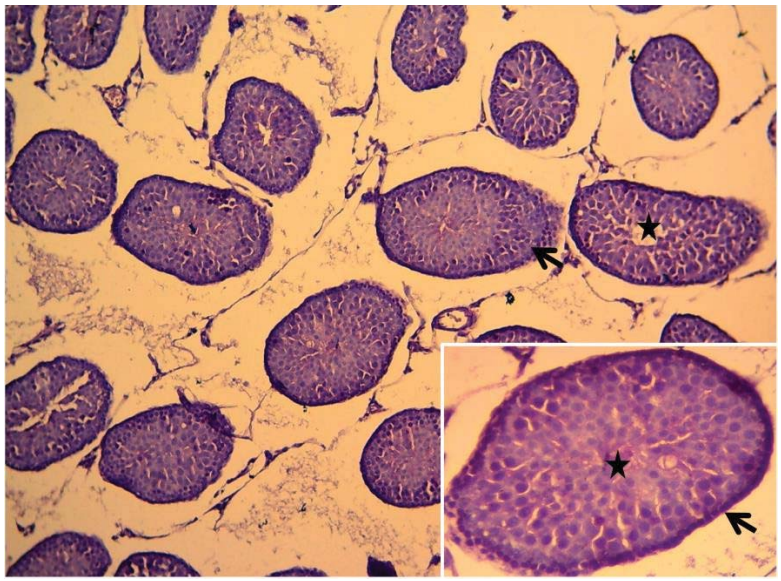


FIGURE 2. Photomicrograph of the transverse section of the testicular tissues of control group. The histopathological section of control group showed normal seminiferous tubules, arrow points to the basement membrane and normal arrangement of germ cell

in basement membrane. Star points to the spermatozoa in the lumen of seminiferous tubules. Main and inset images were stained with H&E, x100 and x400, respectively.

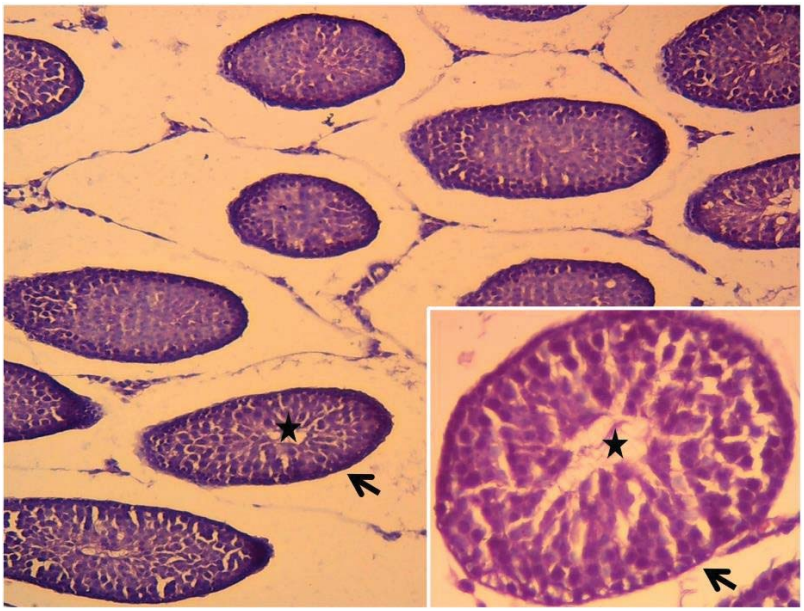


FIGURE 3. Photomicrograph of the transverse section of testicular tissue of sunflower oil group. The histopathological section displayed normal seminiferous tubules, arrow points to the regular arrangement of germ cell in basement membrane. Star points to the spermatozoa in the lumen of tubules. Main and inset images were stained with H&E, x100 and x400, respectively.

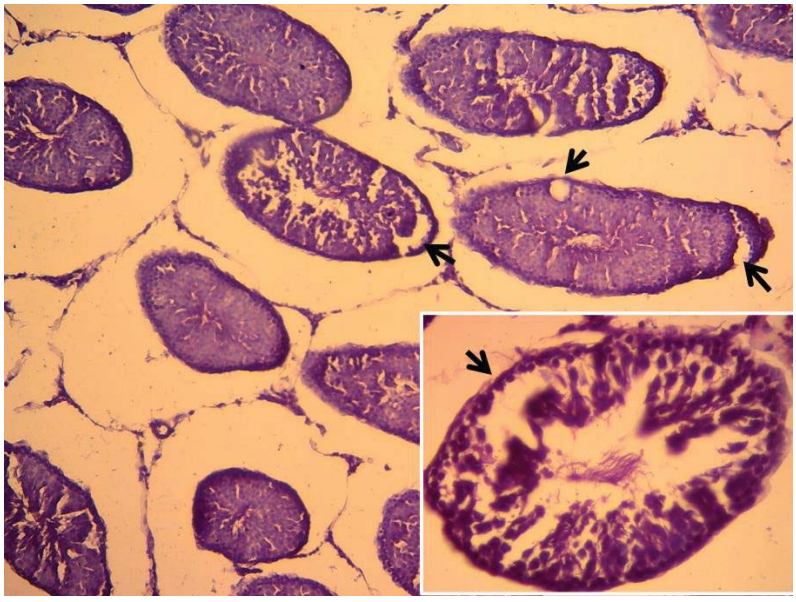


FIGURE 4. Photomicrograph of the transverse section of histopathological alterations of rats in beef tallow group. The histopathological section displayed irregular shape of seminiferous tubules and the arrow points to seminiferous tubules degeneration and vacuoles within the cells. Main and inset images were stained with H&E, x100 and x400, respectively.

DISCUSSION

Recently, the effects of dietary fat composition on human body are gaining more interest. Although there are large numbers of studies that deal with the detrimental effects of dietary fat on human health, little is known about the negative relation between high dietary fat and sperm function. The harmful effects of high dietary fat consumption on human health were demonstrated. Infertility is a complicated health problem having a negative influence on the lives and economy of patients (12). Several studies observed that increase damage of sperm DNA and oxidative stress, is related with diet composition (13,14). Therefore, the current study was designed to investigate the impact of sunflower oil and beef tallow on rat male testicular tissues. The present findings found insignificant increase in the body weight of animals. In term of testis weight, a significant ($p < 0.05$) increase was determined in beef tallow group compared with control and sunflower oil groups. In histopathological investigation of rats that were fed with prepared diets for four weeks, it was observed that the testicular tissues were normal in both control and sunflower oil. In contrast, several histopathological changes were observed in the testicular tissue of rats fed beef tallow-enriched diet. These alterations included degeneration in the seminiferous tubules and reduction in spermatogenesis with lower number of spermatozoa in the lumen of seminiferous tubules. The testes histopathological changes indicated the detrimental effects of beef tallow versus sunflower oil and the possible explanation for the present results is due to the saturated and monounsaturated fatty acid that found in beef tallow. Monounsaturated, polyunsaturated or saturated fat may cause various effects depend on the degree of fat saturation (6). Interestingly, it was observed that the administration of a dietary fat rich in saturated fatty acids caused a reduction in sperm quality and an increase in body weight, hyperinsulinemia, hyperglycemia and dyslipidemia. In contrast, the administration of a dietary fat rich in unsaturated fatty acids can improve the function of male reproductive system (15). Moreover, a number of experiments found that men with high fat diet or obese men reduced sperm quality and quantity, which lead to low fertility rate compared with healthy men (16). It was demonstrated that the excess levels of adipose tissue can increase the amounts of insulin which lead to a reduction in sex-hormone-binding-globulin produced in the liver then increase free estrogen levels and caused alterations in the testosterone and estrogen ratio (17). However, a series of experiments have shown the benefits effects of the vegetable oil compare with animal tallow. It was found that body fat accumulation was lower in rats fed the safflower oil diet than in those fed the beef tallow diet (18). Also, the serum insulin and triacylglycerol concentration was lower in the former (19). The funding from the present study showed the harmful effects of beef tallow fat diet versus sunflower diet on rat male reproductive system.

CONCLUSION

In conclusion, the findings presented here in this study showed that the beef tallow diet significantly increased the testes weight and can induced several histopathological changes in experimental rats. These alterations were included degeneration in the seminiferous tubules, depression of spermatogenesis, detachments of spermatogonia from the basal membrane and presence of vacuoles in the epithelium of seminiferous tubule.

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REFERENCES

1. K. Vijaimohan, M. Jainu, K.E. Sabitha, S. Subramaniyam, Life sciences. 2006;79:448–54.
2. A.O. Hammoud, D.T. Carrell, M. Gibson, C.M. Peterson, A.W. Meikle, Asian J Androl. 2012;14(1):77–81.
3. A.D. Usman, W.F. Wan Ahmad, M.Z.A. Ab Kadir, M. Mokhtar, R. Ariffin, World Appl Sci J. 2012;16(5):656–64.
4. B. Vessby, M. Uusitupa, K. Hermansen, G. Riccardi, A.A. Rivellese, L.C. Tapsell, et al. Diabetologia. 2001;44(3):312–9.
5. J. Donaldson, M.T. Madziva, K.H. Erlwanger, Asian-Australasian J Anim Sci. 2017;30(5):700–11.
6. 6. C. Xiao, A. Giacca, A. Carpentier, G.F. Lewis, Diabetologia. 2006;49(6):1371–9.

7. 7. S.W. Mercer, P. Trayhurn, J Nutr. 1987;117(12):2147–53.
8. 8. Y. Shimomura, T. Tamura, M. Suzuki, J Nutr. 1990;120(11):1291–6.
9. A. Mohammadi Roushandeh, I. Salehi, M. Mortazavi, Iran Biomed J. 2015;19(2):96–101.
10. Anonymous. J Nutr. 1977;107(7):1340–8.
11. L.C. Junqueira, A.L. Mescher, Jonquiere's Basic Histology Text and Atlas. Journal of Chemical Information and Modeling. 2013.
12. J.D. Ring, A.A. Lwin, T.S. Köhler, Asian J Androl. 2016;18(3):357–63.
13. C.D.B. Fernandez, F.F. Bellentani, G.S.A. Fernandes, J.E. Perobelli, A.P.A. Favareto, A.F. Nascimento, et al. Reprod Biol Endocrinol. 2011;9:1–10.
14. H.W. Bakos, M. Mitchell, B.P. Setchell, M. Lane, Int J Androl. 2011;34(5 PART 1):402–10.
15. A. Ferramosca, M. Di Giacomo, N. Moscatelli, V. Zara, uropean J Lipid Sci Technol. 2017;1–22.
16. B.I. Ghanayem, R. Bai, G.E. Kissling, G. Travlos, U. Hoffler, Biol Reprod. 2010;82(1):96–104.
17. L.M. Davidson, K. Millar, C. Jones, M. Fatum, K. Coward. Hum Fertil. 2015;18(3):184–93.
18. T. Matsuo, H. Takeuchi, H. Suzuki, M. Suzuki, Asia Pacific J od Clin Nutr. 2002;11(January):302–8.
19. T. Matsuo, M. Suzuki, J Clin Biochem Nutr. 1994;17:179–88.