

How do vegetable oils (hazelnut and canola) affect the reproductive system in male rats?

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Summary *Objective: Vegetable oils have an important place in our daily diet. This study starts from this point to investigate the effects of canola oil and hazelnut oil in the male reproductive system in rats.*

Material and methods: 30 male rats were used in this 16-week study. The animals were divided into three groups: the animals in group I served as the control group, while the animals in group II and group III were fed with hazelnut and canola oil, respectively. The testes of all rats were excised for histopathologic evaluation and immunohistochemical (IHC) evaluation with a standard method. Blood samples were obtained for determination of serum hormone levels.

Results: No significant differences were noted with respect to behavior or weight among the three groups. Rats in the canola oil group (group III) had higher luteinizing hormone (LH) and higher testosterone levels than rats in the control group. Rats who received hazelnut oil (group II) exhibited similar findings, with these levels being higher than they were in the control group. No statistical differences were shown for histopathology or IHC testosterone antibody levels across all treatment groups. Conclusion: Canola oil was shown to have a greater effect on serum LH and testosterone compared to the control group and the group fed with hazelnut oil. Further investigation is required into how these oils affect serum hormone and sperm activity.

KEY WORDS: Canola oil; Hazelnut oil; Reproductive system; Testosteron.

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INTRODUCTION

Vegetable oils have an important place in our daily diet. Commonly used sunflower and olive oil have been widely used since long time. In recent times it has been able to meet our everyday needs in hazelnut oil and canola oil (rapeseed oil), which are increasingly used in areas where they are particularly grown.

Canola is a plant with bright yellow flowers that belongs to the *Brassicaceae* family. Originally from the Mediterranean area and Northern Europe, *B. napus* is commonly known as rapeseed, and was identified in

2000 BC as a high-erucic acid crop (1). The oil from rapeseed contained > 40% erucic acid, and hesitation existed about this high acid content (as observed in animal studies). High-erucic acid rapeseed oil used to be produced in North America solely in small quantities for industrial, nonfood use (2-3). However, in 1976, Canadian scientists were able to improve the quality of previous cultivars of rapeseed by growing the plant traditionally, yielding a conversion that allowed commercial consumption. In 1979, Canada registered the word "canola" to describe a new seed found to yield an oil; this oil included a smaller amount of erucic acid and glucosinolates. Inherently, canola has specific cut-off levels of erucic acid (< 2%) and glucosinolates (< 30 $\mu\text{mol/g}$) for consumption both in humans and in animals (4).

In 1985, the United States Food and Drug Administration (FDA) accepted canola oil as "generally recognized as safe" (GRAS) as a dietary component (5). Canola has become one of the most important oilseed crops worldwide over the past 40 years; currently, canola oil is the third-largest vegetable oil by volume, after palm and soybean oil (3). Hazelnut (*Corylus avellana* L.) is a well-recognized tree nut worldwide. Hazelnuts are mainly produced in Turkey, Italy, Spain, the USA, Portugal and France. Hazelnut oil includes a high amount of both monounsaturated and polyunsaturated fatty acids as well as tocopherols (6).

Hazelnut oil contains 74.2%-83.1% oleic acid and linoleic acid. Therefore, the incorporation of hazelnut oil in meat products may have favourable effects on the health of consumers (7). Oils have an important place in the structure of the reproductive hormone system. The main hormones of the male reproductive system are mainly testosterone with follicle stimulating hormone (FSH) and luteinizing hormone (LH).

There are very few studies on the effects of these oils on the male reproductive system. Because of that; the aim of our study is to investigate the effects of canola oil and hazelnut oil on the male reproductive system, with a focus on evaluating the effects on serum hormone levels and testis histopathology.

No conflict of interest declared.

MATERIALS AND METHODS

The selection and preparation of animals

In this study, 30 four-month-old Sprague-Dawley rats were used. Inonu University Faculty of Medicine, Experimental Animal Research and Production Center provided the rats for study subjects. During the four-month study period, the average weight of the rats was 236.71 ± 19.18 gr. The study was executed according to the rules of the National Health Institute at Inonu University Experimental Research Laboratory, and was performed with the consent of the Animals Ethics Committee of the Inonu University Faculty of Medicine Ethics Committee (2011/05/18). Animals were sheltered in groups of five in standard-sized (40 by 60 cm) cages. The subjects were given 7 days to adapt to their new environment, then separated into 3 groups of 10 rats. Specially prepared hazelnut oil (12% concentration) and canola oil (12% concentration also), along with the standard food containing dry pellets and tap water, was used as the diet. Indoor lighting was tuned for 12 h of light and 12 h of darkness. Heat and moisture were set to $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $50\% \pm 10\%$, respectively.

Preparations for the experiment

After the animals were separated into groups, each group was divided into half and sheltered in 2 separate cages. Any additional process was applied to the first group (Group 1); the second group was fed with the 12% hazelnut oil added to the food (Group 2). The third group was fed with food that included 12% canola oil (Group 3). External factors often have an impact on testicular functioning. The photoperiod is one of these factors: long photoperiods increase testicular functioning, while short periods reduce it (8). In order to avoid any morphological changes among the groups caused by the various light amounts, all rats were exposed to the rotating 12-h light/12-h dark environment in the lab. Reproduction of sperm gradually increases up to the 75th day and the testicular weight increases up to the 100th day. When the first spermatozoa were found at the epididymis tail, about when the rats were 50 days old, they were considered adult (mature) (9). All of the rats used in our experiment were fed for approximately 4 months so that they would become 6-month-old adults and have the highest sperma reproduction. The intent in this design was to eliminate the variance in the sperma reproduction and the epididymal spermatozoa number caused by age.

Preparation of the food

As in the previous studies, the food was prepared considering the daily oil need. Pellet food was supplemented with the 12% oil (canola or hazelnut) (8-10). It was used to feed rats after the addition of appropriate fat to the amount of feed and control of homogenous distribution. The total absorption was ensured and, after a checking process, daily food of 18-24 g/rat was placed into the food reservoir. In order to keep the food fresh, a small amount of new food was prepared every week and the drinking water was provided via fresh tapwater ad libitum.

Biochemical method of analysis

The blood taken for the biochemical analysis was put into

the tubes and centrifuged at 3500 rpm for 15 minutes. The obtained serum was put into separate tubes and numbered for use with each group. Serum samples were evaluated using previously provided ELISA kits specific for rats that measured FSH (Cusabio Biotech Co., Ltd), LH (Cusabio Biotech Co., Ltd), and Testosterone (DRG International, Inc, United States) with the Basic Radim Immunoassay Operator (BRIO) (Radim spa, Pomezia, Italy) device.

Histopathological and immunohistochemical research method

After separation by surgical dissection, the testicles were placed in Bouin's fixative for histopathological evaluation. The testis tissue was chopped with 2-mm apertures using microtome knives; routine tissue observation was practiced by sectioning one slice. Five-micron sections were derived from the paraffin-embedded blocks formed from tissue samples. After the process of deparaffinization, the sections were dyed with hematoxylin-eosin (H-E). In the course of histopathological evaluation, the architectural structure was examined by the help of magnifying method beforehand. Subsequently, the size and the number of the seminiferous tubules, the thickness of the tubule basal membranes, the relative share and types of germ cells in the seminiferous germs, the degree of interstitial fibrosis, and the existence of the Leydig cells are evaluated in the course of a general examination of the testis cross-section. The evaluation was standardized by using the quantitative Clinical Scoring Method of 1 to 10 for each seminiferous tubule as suggested by Johnsen (11). Four-micron sections were obtained and placed onto polysine slides for immunohistochemical dyeing, which was performed automatically using the Lecia Bond Max (Leica Microsystems Inc. U.S.A) device. Testosterone primary antibody (GeneTex, USA) was used as the primary antibody. The strength of the testosterone antibody dyeing was evaluated as a semi-quantitative method; the absence of dye was assigned zero points, while the most powerful dyeing was assigned a score of 3 points.

Statistical method of analysis

SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois) was used for statistical analysis. The Kolmogorov-Smirnov test was used to measure the correspondence of the different parameters and the normal distribution curve. A correspondence was observed between the LH, testosterone, and Johnsen scores as well as between the rats' pre-experimental and post-experimental testicular weights and the normal distribution curve ($P > 0.05$). On the other hand, no correspondence existed between the FSH hormone distribution and the normal distribution curve ($P < 0.05$). The Kruskal-Wallis, one-way analysis of variance (ANOVA), and post-hoc Tukey tests were used for statistical analyses in the groups that corresponded with the normal distribution curve. $P < 0.05$ was assumed to be significant for all evaluations. Values are given as average \pm standard deviation (mean \pm SD).

RESULTS

All of the rats were kept alive till the end of the experiment. Rats' pre-experiment and post-experiment (after 16

Groups→	Control group AO ± SS	Hazelnut group AO ± SS	Canola group AO ± SS	P
Pre-experiment average weight (g)	247.2 ± 23.10	228.10 ± 34.00	252.50 ± 50.46	0.327
Post-experiment average weight (g)	325.0 ± 32.47	316.00 ± 27.39	345.50 ± 43.19	0.177

Table 1.
Increase in the weights of the rats after 16 weeks of feeding.

Groups↓ hormones→	LH (mIU/ml) AO ± SS	Testosterone (ng/ml) AO ± SS	FSH (Min-Med-Max)
Control	10.86 ± 5.47	1.29 ± 0.45	12.97 - 26.38 - 77.24
Hazelnut oil	13.68 ± 3.99	1.88 ± 0.68	21.74 - 30.90 - 38.44
Canola oil	14.95 ± 4.55	2.53 ± 0.74*	19.06 - 31.91 - 9.33

Table 2.
Average values of serum hormone LH and testosterone * (P = 0.001).

Groups	Johnsen Score Averages (AO ± SS)
Control Group	9.37 ± 0.27
Hazelnut Group	9.21 ± 0.31
Canola Group	9.26 ± 0.20
Total	9.28 ± 0.25

Table 3.
Johnsen Testicle Biopsy Scores for each group and group averages (P = 0.362).

weeks of feeding) average weights are given in Table 1. No variance existed between the groups' initial weights or with these weights during and at the end of the experiment (P > 0.05).

The average wet weight of the removed testicles was measured as 1.37 ± 0.80 g for the control group, 1.34 ± 0.13 g for the hazelnut oil group, and 1.48 ± 0.21 g for the canola oil group; there was no significant difference between any 2 of the 3 groups for this parameter (P = 0.103). Furthermore, no significant statistical difference existed in the levels of serum FSH among the groups (P > 0.05) (Table 2).

The levels of serum LH for rats fed with either hazelnut or canola oil did increase, but this increase was not statistically significant (P > 0.05). When the levels of serum testosterone of the 3 groups were compared, there was a statistically significant increase in the canola group (P < 0.05). The slight increase in the hazelnut group was not significant (P > 0.05) (Table 2).

Histological examination of the testis

The testis were removed for histopathological analysis

and preserved within the 10% Bouin's fixative. The testicular parenchyma of the rats (covered by tunica albuginea, Leydig cells, and interstitial connective tissues) were examined in the seminiferous tubules and interstitial.

In the histopathological analysis, 10 cross-cut seminiferous tubules were randomly scored 1 to 10 for each rat testicle according to Johnsen scoring criteria. After that, the average value for each rat was calculated, with total group scores and averages obtained (Table 3).

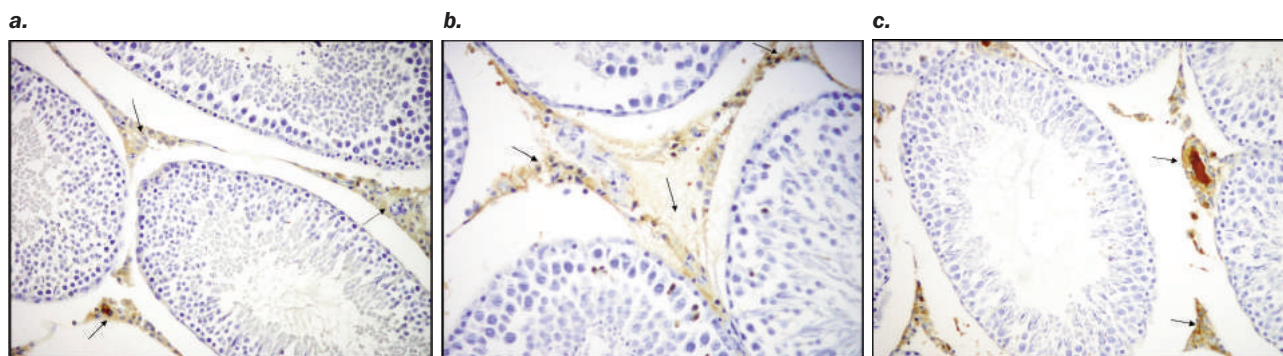
No significant difference was found among the Johnsen score averages (P > 0.05). Furthermore, no significant difference existed between the immunohistochemical dyeing strength of total tissue testosterone antibody of the 3 groups (Figure 1).

DISCUSSION

Oils are one of the most significant nutrients in that they have essential roles in the human diet and conduct vital activity in the body. The positive or adverse effects of canola and hazelnut oil on the body have not been precisely determined, although their consumption have

Figure 1.

Sample of interstitial painted testosterone antibody group and scores.
 a) Immunohistochemical staining intensity canola oil group 20X (Score 3).
 b) Immunohistochemical staining intensity hazelnut oil group 40X (Score 2).
 c) Immunohistochemical staining intensity control group 20X (Score 1).



been continuously increasing in people's diets worldwide. Limited and not conclusive scientific evidence would suggest some benefit for *canola* oil consumption, but results from studies implementing diets containing *canola* oil in experimental animal models have provided us with conflicting data (10-12).

A study by *Okuyama and colleagues* using steroid hormones, *canola* and soybean oils for a 3-month period in hypertensive rats who were prone to stroke compared various elements in the rats at the end of the 3-month period, although they did not evaluate the impact of the 2 oils on the reproductive system. As a result, while the testosterone values measured in the testicles of rats in the *canola* group were found to be low compared to those in the soybean group, corticosteroid and estradiol levels in the tissues demonstrated no significant difference. This difference was assumed to arise from a pathophysiology found in rats with hypertension (13). In our study, serum testosterone level increased while testosterone antibody values measured in the test were similar to the control group.

Multiple other studies exist on the effects of *canola* and other oils over periods ranging from 3 to 7 months. Infants fed with and without *canola* oil from the ages of 4 weeks to 7 months did not exhibit significant differences in height and length based on whether they consumed the oil as part of their diets (14). Similarly, in our study, weight difference was not shown in rats fed with *canola* oil and *hazelnut* oil after 4 months period.

de Almeida and colleagues evaluated the effects of a diet containing *canola* oil on the morphology of seminiferous tubules of young rats (15).

With evaluation of FSH, LH, and testosterone levels, significant and important information was found in terms of the determination of potential malfunctioning of the reproduction pathophysiology and the conditions of the hypothalamus-hypophyseal axis.

In our study, serum testosterone levels in the groups receiving *canola* oil were higher compared to the control group ($P < 0.05$). Higher levels were also found in the *hazelnut* group, although it was not statistically significant compared to the control group ($P > 0.05$).

No significant differences were found among the 3 groups in terms of testicular and body weight ($P > 0.05$). FSH, prompted by the hypothalamus and released by the anterior pituitary gland, stimulates sertoli cells in the seminiferous tubules, speeding up sperm production of spermatids. Furthermore, it was effective in the development and maintenance of FSH sufficient testicular function in men. Within our study; no significant difference was found among the FSH values of the 3 groups ($P > 0.05$).

LH, prompted by the hypothalamus (GnRH) and released by the anterior pituitary gland, stimulates the release of testosterone in the interstitial Leydig cells. In our study, LH levels in the *hazelnut* and *canola* oil groups were found to be higher compared to the control group, although it was not assumed to be statistically significant ($P > 0.05$). These results can be attributed to the assumption that these kinds of oils can enhance the stimulation of GnRH via the effects from the hypothalamus or that the oils can generate a slight LH stimulus by

directly influencing the anterior pituitary gland. The increased level of testosterone, especially in the *canola* group fed with the oils supplied with direct LH, indicates that the results may be explained by the actions of the hypothalamus-hypophyseal axis. New experimental studies are needed to fully understand the effects of the hypothalamus-hypophyseal way. The effects of testosterone on erectile dysfunction and libido indicated that testosterone has a considerable contribution to maintenance of libido and sexual function (16). The fact that these oils have no significant effect on FSH compared to the control group increases the probability that these hormones are more effective in exerting their effects coming from the hypophysis as opposed to the hypothalamus. There are not many publications examining the effects of these oils on the reproductive system.

The effects of *hazelnut* and *canola* oil on testicle histopathology were not indicated beforehand. Histopathologic examination of the 3 groups' testicular tissue did not reveal significant differences according to evaluation using Johnsen scoring ($P > 0.05$).

Thus, no pathological adverse events related to use of these everyday oils in the diet were shown in this study. Furthermore, no statistically significant differences between the 3 groups were revealed as a result of the semi-quantitative immunohistochemical evaluation of testicle-tissue dyeing intensity ($P > 0.05$).

CONCLUSION

The effects of vegetable oils, which we often use in our daily life, on the reproductive system can be affected by various mechanisms by affecting the hormones. Additional studies are needed for the determination of the exact effect of this condition on the tissues and its influence on spermiogenesis.

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