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Short term effects of genistein on the testes of quail (*Coturnix coturnix*)

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Abstract: Although there have been a number of studies on the effects of phytoestrogens, especially genistein, on testes, its effects on the testis tissue of birds have not been studied yet. The present study aims to reveal the effects of genistein on quail testes through the use of histological and immunohistochemical methods. In the study, a total of 64 male quails (*Coturnix coturnix*) were used, each of which was 50 days old. Three different doses of genistein were administered by gavage to the separate groups at intervals of 15 and 30 days. According to the results of the histological analysis and DNA in situ nick end labeling (TUNEL), it was detected that high doses of genistein, especially in 30-day applications, caused more damage compared to the control group and the group receiving 15-day applications in terms of histology and sperm number. Moreover, it was also detected in the 15-day and 30-day application results that both low and high doses of genistein induced apoptosis.

Key words: Quail, genistein, testis, apoptosis

Genistein'in bıldırcın (*Coturnix coturnix*) testisi üzerine kısa süreli etkisi

Özet: Fitoöstrojenlerin özellikle de genisteinin testis üzerindeki etkisi ile ilgili bir çok çalışma olmasına rağmen kuşların testis dokusu üzerindeki etkisini gösteren bir çalışma henüz bulunmamaktadır. Çalışmamızda histolojik ve immunohistokimyasal yöntemler kullanılarak bıldırcınların testis dokusu üzerine genisteinin göstermiş olduğu etkinin açığa çıkarılması amaçlanmıştır. Çalışmada 64 adet 50 günlük erkek bıldırcın (*Coturnix coturnix*) kullanılmıştır. Genisteinin üç farklı dozu 15 gün ve 30 gün süreyle ayrı gruplara mide sondası yardımıyla ağızdan verildi. Histolojik inceleme ve in situ DNA uç işaretlemesi (TUNEL) sonuçlarına göre genisteinin yüksek dozda ve özellikle 30 günlük uygulamalarında histolojik ve sperm sayısı bakımından kontrole ve 15 günlük uygulamalara göre daha çok hasara neden olduğu, ayrıca hem düşük hem de yüksek dozlarının 15 günlük ve 30 günlük uygulama sonuçlarında apoptozu teşvik ettiği belirlenmiştir.

Anahtar sözcükler: Bıldırcın, genistein, testis, apoptoz

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Introduction

Due to their high protein content, quails are species that have been in high demand lately, since both their meat and eggs are capable of meeting food requirements (1,2). Legume grains and soy products serve an important role as animal feeds used in the raising of poultry, including quails (3). Legumes and soy products contain a group of chemicals known as phytoestrogens. Phytoestrogens may have estrogenic and antiestrogenic effects, especially on the reproductive system, by showing structural similarity to the hormone estradiol (4,5). Genistein is one of the most common non-steroid (6) phytoestrogens present in soy products, soy beans, and other legumes (7,8).

Genistein can show both estrogenic and antiestrogenic effects, competing with estradiol at the receptor level (9). It is also known that genistein stops the cell cycle at the G_2-M phase (10) and induces apoptosis in testis cells (11).

In the present study, the effects of genistein on testis histology and testicular cell apoptosis in quails were studied using light microscope and DNA in situ nick end labeling (TUNEL). This study aims to reveal new data that will contribute to our understanding of the relationship between nutrition and the reproductive biology of birds by comparing the results of the present study with similar studies of other birds and mammals.

Materials and methods

In this study, a total of 64 male quails (*Coturnix coturnix*) were used, each of which had reached a sexual maturity of 50 days. Each of the experimental groups which received genistein (provided by Mikro-Gen Medicine Ind. Inc.) consisted of 8 quails, as did the control group. The genistein was administered by gavage. The experimental groups and exposure amounts of genistein were as shown in Table.

Histometric and histological methods

The testis tissues from the animal groups shown in the Table were fixed in Bouin's solution for 24 h at 20 °C and embedded in paraffin wax. The sections of 5 μm were stained with hematoxylin and eosin following the method described by Humason (12). The diameters of the seminiferous tubules and the germinal epithelial height of the control and experimental groups were evaluated using the standard point counting method described by Attnassova et al. (13).

The DNA in situ nick end labeling (TUNEL) method

After the testis tissues from the groups shown in the Table were fixed in 10% buffered formalin, preparates were prepared using histological methods. The cells with free DNA ends were marked using a Roche brand in situ cell death detection kit, POD, and stained with Gill's hematoxylin.

Statistical analysis

In the analyses conducted on the experimental animals, the differences in testis weights, seminiferous

Table. Control groups and experimental groups that were given genistein.

15 DAYS		30 DAYS	
Control group I	Only corn oil	**Control group II**	Only corn oil
Experimental group I	0.5 mg/kg	**Experimental group IV**	0.5 mg/kg
Experimental group II	25 mg/kg	**Experimental group V**	25 mg/kg
Experimental group III	50 mg/kg	**Experimental group VI**	50 mg/kg

tubule diameters, germinal epithelial heights, and apoptosis indexes between the control and experimental groups were analyzed through single direction variance analysis (ANOVA). The value of $P < 0.001$ was considered to be statistically significant. In addition, the differences between findings of the 15-day and 30-day experimental groups were evaluated statistically using the Scheffe's method of variance analysis. A value of $P < 0.05$ was considered to be statistically significant.

Results

As a result of the macroscopic analysis, the average testis weight for each group was determined and results are shown in Figure 1. In terms of testis weight, compared to the control group I, experimental groups II and III showed a significant increase. When experimental group V and experimental group VI were compared to control group II, a significant increase was detected in testis weight ($P < 0.001$). Furthermore, when experimental group I and experimental group IV, experimental group II and experimental group V, and experimental group III and experimental group VI were compared to each other, a significant ($P < 0.05$) increase was observed in testis weight.

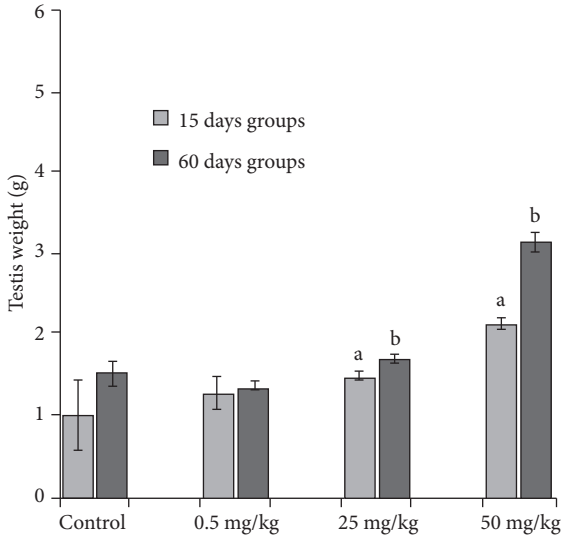

Figure 1. Testis weights for control and experimental groups (a, b: $P < 0.001$).

When seminiferous tubule diameters were evaluated, a significant ($P < 0.001$) decrease was observed in experimental group III as compared to control group I while a significant increase ($P < 0.05$) was observed between experimental groups III and VI. The values recorded for the other groups were not found to be significant.

When germinal epithelial heights were evaluated in control group I and in experimental groups I, II, and III, the germinal epithelial heights were found to show a significant decrease ($P < 0.001$) compared to the control group. In experimental group VI, germinal epithelial height increased significantly ($P < 0.001$) compared to control group II. Experimental group V also increased significantly ($P < 0.05$) in comparison to experimental group II. In experimental group VI, a significant increase ($P < 0.05$) was observed in germinal epithelial heights when compared to experimental group III.

The findings for experimental groups given genistein for 15 days were examined and when results from experimental groups I, II, and III were compared with control group I, it was observed that the seminiferous tubules and germinal epithelial cells maintained their regular structure. It was also seen that in experimental groups I and II, the density and morphology of the spermatocytes in the seminiferous tubule lumens were similar to those of the control group; in experimental group III, however, a decrease in the density of spermatocytes was detected (Figure 2). A look at the findings of the experimental groups given genistein for 30 days showed that when experimental groups IV, V, and VI were compared with control group II the regular structure of the seminiferous tubules was maintained. Intercellular spaces were identified in some parts although germinal epithelial cells were in order. In experimental groups IV, V, and VI, the morphology of the spermatocytes in the seminiferous tubule lumens was similar to that of the control group. However, while the spermatocyte density in experimental group IV was similar to that observed in the control group, this density was seen to decrease in experimental groups V and VI when compared to the control group (Figure 3).

When the groups that were given genistein for 15 days were compared with control group I,

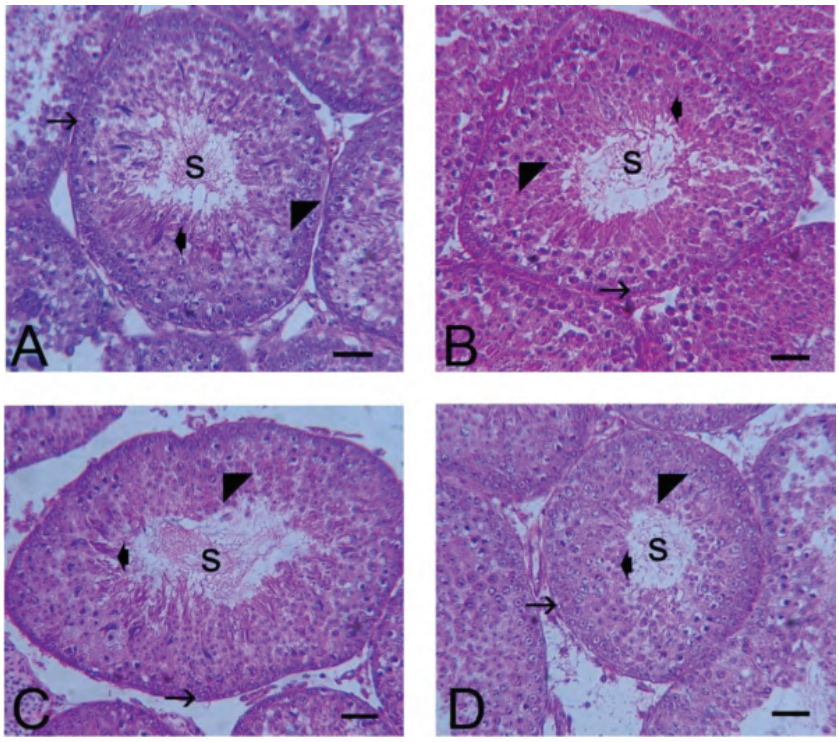

Figure 2. Histological sections of quail testes from control groups and experimental groups that were given genistein for 15 days. (H&E, bar = 13 µm). Spermatogonium (→), primary spermatocyte (◢), spermatid (⧫), sperm (S). A. The control group; B. The group given 0.5 mg/kg genistein; C. The group given 25 mg/kg genistein; D. The group given 50 mg/kg genistein.

it was observed that the proportion of apoptosis increased significantly in all groups ($P < 0.001$). Similarly, in the groups which were given genistein for 30 days, a significant increase ($P < 0.001$) in the proportion of apoptosis was observed in all groups compared to control group II. Furthermore, when experimental group I and experimental group IV, experimental group II and experimental group V, and experimental group III and experimental group VI were compared with each other, it was detected that the apoptosis proportion in these groups also increased significantly ($P < 0.05$) (Figure 4).

Discussion

In studies conducted to determine the effects of genistein on testis tissue, testis weight, seminiferous tubule diameter, the histological structure of the testis, and the amount of apoptosis seen in the testes have all been considered (13-18). Genistein, which is a phytoestrogen, shows various effects on the testes by binding estrogen receptors, interfering with sex steroid production, inhibiting protein kinases, preventing cell proliferation, and decreasing the production of sex steroid binding proteins (19).

Magee (20) has reported that the administration of 0.1% genistein and 0.5% genistein had no significant effect on testis weight when compared to a control. In a study from 2001, however, Nagao et al. (14) showed that testis weight increased significantly in rats given 25, 50, or 100 mg/kg of genistein in comparison with the control group.

A study undertaken by Fritz et al. in 2003 (15) determined that there was no increase in the testis weight of rats that were given 25 mg/kg or 1000 mg/kg genistein for 35 days. In another study in the same year (16), 10 mg/kg and 40 mg/kg genistein were given subcutaneously to rats for 3 weeks; it was found that in the group that was given 10 mg/kg genistein the testis weight did not change compared to the control group, although in the group which was given 40 mg/kg, the testis weight decreased compared to the control group. In a 2004 study by Adachi et al. (17),

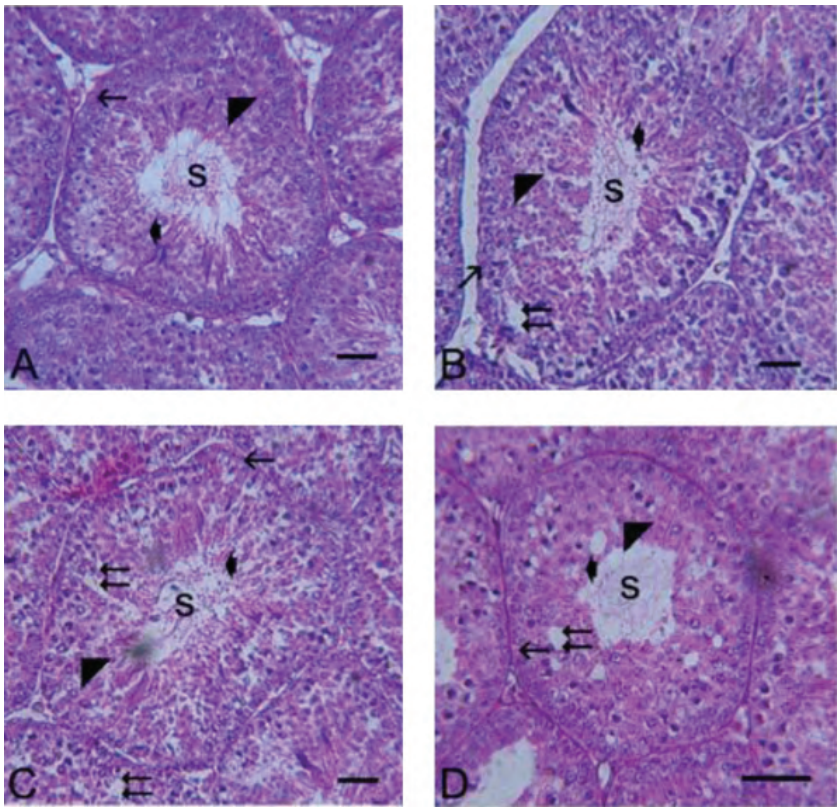

Figure 3. Histological sections of quail testes from control groups and experimental groups that were given genistein for 30 days. (H&E, bar = 13 µm). Spermatogonium (→), primary spermatocyte (◢), spermatid (⧫), sperm (S), expansion the intercellular spaces (⇆). A. The control group; B. The group given 0.5 mg/kg genistein; C. The group given 25 mg/kg genistein; D. The group given 50 mg/kg genistein.

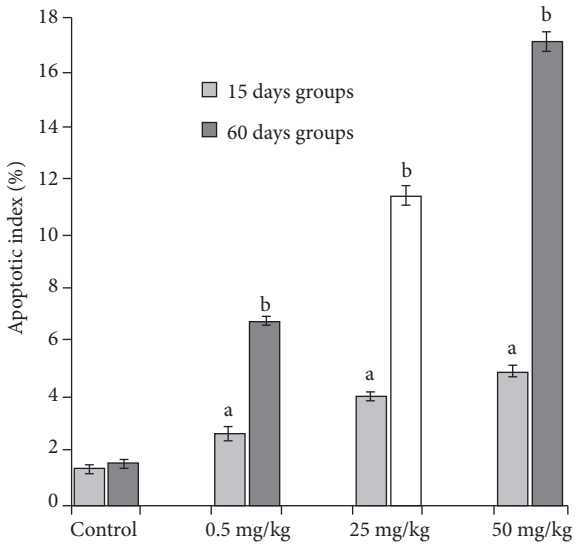

Figure 4. The apoptotic index (%) for control and experimental groups (a, b: $P < 0.001$).

1 mg/kg genistein was given to newborn rats from the first day of birth until the fifth day. When the rats were 12 weeks old, the differences that occurred in their testes were analyzed. It was found that there was no difference in the testis weight of the rats in the group given genistein compared to the control group. In a study by McClain et al. (18), 7-week-old rats were acute, subchronically, and chronically given genistein orally. They found that in the rats which were exposed to acute and subchronic genistein for 4 weeks, the testis weight was not affected while in the rats exposed to subchronic genistein for 13 weeks the testis weight increased in correlation with the genistein dose given compared to the control group. In the chronic experiment, the rats were exposed to 50 mg/kg and 500 mg/kg genistein for 52 weeks. It was found that in these rats the testis weight also increased in correlation with the genistein dose

given compared to the control group. According to the present study, a significant increase was seen in the groups that were given 25 mg/kg or 50 mg/kg genistein, whereas there was no significant increase in the testis weight in the group that was given 0.5 mg/kg genistein when compared to the control group. In a study by Aktaş et al. (21), White Leghorn eggs were exposed to 500 µg/egg genistein during embryonic development. They found that genistein did not have a negative effect on embryonic testis development. These findings indicate that, similar to the results obtained for other animal groups, low doses of genistein do not cause any change in the testis weight of birds although higher doses increase the testis weight.

In a 2003 study conducted by Fritz et al. (15) on rats, it was shown that genistein did not change the seminiferous tubule diameter significantly. According to the results of the present study, there was a significant decrease in tubule diameter in the group that was given 50 mg/kg genistein for 15 days; on the other hand, there was a substantial increase in the seminiferous tubule parameter if genistein was administered for 30 days. It is thought, however, that this increase was not related to the natural structure of the tissue and sperm production, being caused instead by the intercellular spaces formed in the germinal epithelial tissue. No important change was observed in any of the experimental groups with regard to the seminiferous tubule histology when compared to the control group (14,16,17) and it was seen that spermatogenesis continued normally (17).

In the present study, when all of the experimental groups were evaluated in terms of sperm morphology and compared to their respective control groups, the significant decrease in the groups that were given 50 mg/kg genistein for 15 days and 25 mg/kg or 50 mg/kg genistein for 30 days suggests that an overdose of genistein decreases the density of sperm in quails as well as in rats (22).

In previously undertaken studies, it has been stated that genistein had an apoptotic effect on spermatogenic cells found in the testes and that this effect was related to the dose administered (11,13,23). In the present study, in all of the groups that were given genistein for 15 or 30 days, the apoptosis index increased significantly in correlation with the dose when compared to the control group.

In conclusion, this study showed that genistein, one of the most dominant phytoestrogens found in soy, soy products, and other legume species used in the feeding of poultry, induces apoptosis in correlation with the dose in the testes of quail, one of the best representatives of poultry.

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