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Effects of cottonseed flour on testicular structure and sperm motility of rats

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Summary

This study was undertaken to investigate the effect of cottonseed flour intake on sperm motility and pathological changes in testicular tissue in male rats. A total of 100 male rats were divided into 5 groups and each group was fed with a diet containing cottonseed flour at a level of 0% (control), 5%, 10%, 20%, and 40% for 80 days. Sperm motility of the rats and histopathological examination of their testicles were determined on days 20, 40, 60, and 80. Results indicated that sperm motility in rats fed with a diet containing 10%, 20%, and 40% cottonseed flour was significantly decreased. Clinical symptoms were evident only in rats fed with 40% cottonseed flour. Histopathological changes of varying severity in the testicular tissue of rats occurred only in those fed with 10%, 20%, and 40% of cottonseed flour. These changes included a decline in numbers of germinal layers, degeneration, desquamation, vacuolization of germinal epithelium, formation of intratubular giant cells, connective tissue proliferation in the intertubular area, and vacuolization of some Leydig cells. The results of this study indicate that adding cottonseed flour exceeding a level of 5% to the diets of rats decreased sperm motility and caused pathological changes in testicular tissue.

Keywords: Cottonseed flour, testicle, sperm motility

Cottonseed and its by-products are common feed supplements for livestock rations. However, cottonseed and cottonseed by-products contain gossypol, a yellow, polyphenolic glycoside and that has been known to have cardiotoxic and hepatotoxic effects (11). Toxic effects of glycoside in pre-ruminants and monogastric animals have been reported by many researchers (10, 11, 14, 22, 24). In addition to its cardiotoxic and hepatoxic effects, it has been reported that gossypol may cause infertility in male animals and human (12-14, 19). Gossypol was mostly used as its extract in those studies indicating its association with infertility in males (2, 5, 6, 12). However, these conclusions might be somewhat misleading since gossypol intake by livestock generally occurs through consumption of cottonseed in its natural form rather than in extracted form. For instance, adding cottonseed to cattle ration in its natural form is a common practice in developing countries. There are very limited data on that effect of gossypol was investigated by feeding animals with rations containing cottonseed (10) and/or cottonseed flour (20). Therefore, objective of the present study was to investigate the effect of consumption of cottonseed flour (CSF) at various levels for various periods on pathological changes in testis and on sperm motility of male rats.

Material and methods

A total of 100 male-Wistar rats were used. The rats were 3.5 months old and weighed approximately 350-400 g. The animals were fed with a standard diet for 10 days and then divided into 5 groups at random, as 20 rats per group. Animals in each group were housed as 5 animals per cage and subjected to daily lightening regime of 14 h light followed by 10 h dark. Cottonseed flour was produced by separation of seed content from outer layer by cracking followed by sifting. The resulting flour was added to the diet of rats at levels of 0% (Group I, control), 5% (Group II), 10% (Group III), 20% (Group IV), and 40% (Group V) before mixing and making pellets. The rats were fed with these pellets for 80 days. Feed and water were provided ad libitum through out of the study. Five rats from each treatment group were selected randomly on days of 20, 40, 60, and 80. These rats were decapitated by cervical dislocation. Sperm motility of rats was determined as described by Hafez (4). Testicles were removed and weighed. Size of testicles was determined using a caliper. For histopathological examination, testicle samples were taken and fixed in 10% neutral buffered formalin, processed routinely and stained with hematoxylin-eosin (H&E). A ratio of injured tubules in each sample was determined by counting total and injured tubules (decline in numbers of germinal layers, degeneration, desquamation, vacuolization of germinal epithe-

lium, formation of intratubular giant cells) in 10 randomly selected microscopic fields under a light microscope with 10X magnification (approximately 70-80 tubules).

The contents of cottonseed flour used in this study were analyzed according to AOAC tecniques (1). The free gossypol concentration of cottonseed flour used was determined by spectrophotometer in Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ankara, Turkey.

Statistical analysis of the data was performed using SPSS software package (21). Sperm motility ratios were compared by Chi square test. Size and weight of testicles were analyzed by Variance analysis test. Variance analysis and Chi square tests were performed on injured tubule ratios.

Tab. 1. Effect of dietary cottonseed flour on sperm motility of male rats (%)

Treatment	Days							
	20		40		60		80	
Groups	< %60	> %60	< %60	> %60	< %60	> %60	< %60	> %60
Control (I)	0^{a}	100	0^{a}	100	0^{a}	100	0^{a}	100
%5 (II)	0^{a}	100	0^{a}	100	0^{a}	100	0^{a}	100
%10 (III)	20^{bx}	80	40^{by}	60	40^{by}	60	100^{bz}	0
%20 (IV)	40^{cx}	60	60^{cy}	40	40^{bx}	60	100^{bz}	0
%40 (V)	60^{dx}	40	80^{dy}	20	100^{cz}	0	100^{bz}	0

Explanations: a, b, c, d – numbers within the same column with different superscript are significantly different ($p < 0.001$); x, y, z – numbers within the same row with different superscript are significantly different ($p < 0.001$)

Tab. 2. Effect of dietary cottonseed flour on injured tubule ratios (%) in testicle of male rats*

Days	Treatment Groups				
	Control (I)	% 5 (II)	% 10 (III)	% 20 (IV)	% 40 (V)
20	0.00^{w}	0.00^{w}	0.75^{ax}	5.00^{ay}	18.70^{az}
40	0.00^{w}	0.00^{w}	1.75^{bx}	11.00^{by}	44.00^{bz}
60	0.00^{w}	0.00^{w}	3.50^{cx}	29.50^{cy}	51.50^{cz}
80	0.00^{w}	0.00^{w}	7.50^{dx}	37.75^{dy}	73.75^{dz}

Explanations: * Injured tubule ratios = (Injured tubules/total numbers of tubules) × 100; a, b, c, d – numbers within the same column with different superscript are significantly different ($p < 0.001$); w, x, y, z – numbers within the same row with different superscript are significantly different ($p < 0.001$)

Results and discussion

Analysis of CSF indicated that 95.64% of dry matter, 38.86% of crude fat, 31.8% of crude protein, 9.16% of crude fiber, 5.57% of ash and 12.77% of nitrogen free extract were found. The free gossypol concentration of CSF used was determined as 12820 ppm.

No clinical symptom or mortality was observed in group I, II, III, and IV during the 80 day period while a total of 4 rats, 1 rat on each of days 28, 56, 67, and 73, died in group V. Clinical symptoms were observed in rats in group V starting from day 20 and manifested as decrease in feed and water intake, bristling, slightly back hunching, and indolence.

No macroscopical lesion was observed in necropsy of animals from group I, II and III during entire experimental period. Compared to control group (group I) rats, more paleness and softness in cut surfaces of the testicles were observed in some animals from group IV and V as of day 60 and 20, respectively. Findings of effect of CSF on sperm motility of rats are presented in tab. 1. Sperm motility of rats in group I and II was over 60% during the entire study period. In contrast, sperm motility of rats in group III, IV, and V decreased as the CSF level increased in their diet. Sperm motility of rats in group IV and V was determined as 0% on days 80 and 60, respectively. There was no significant difference in weight and size of testicles between treatment groups at the sampling times. However, significant differences were observed in injured tubules ratio among the groups (tab. 2). Similar to sperm motility, no injured tubule was found in testicles of animals from group I and II while an increased level of tubular injury of animals were found in other groups as the CSF increased in their diets.

There was not any histopathological changes in group II and I whereas histopathological findings of an increasing severity by the time and level of CSF in the diet of rats were observed in other treatment groups. More specifically, pathological changes were located in only seminifer tubules in group III and in both seminifer tubules and intertubular areas in group IV and V. The microscopical effect of the cottonseed flour on the testes was not uniform; both injured and normal seminiferous tubulus were observed in the same section. There was an exfoliation of germinal cells from the epithelium into the lumen of the tubulus. Degeneration, necrosis, and disorganization were observed in germinal epithelium. In addition, numbers of cell layers were reduced (fig. 1). The tubulus shown intraepithelial vacuolization (fig. 2), and the lumen of the tubulus were filled with debris of germ cells and spermatozoa. On day 80 in group IV and on day 60 in group V, degenerative changes in the affected tubulus were further pronounced. In these groups, injured tubules were covered with only a thin layer of Sertoli cells. Spermatogonia, spermatocytes, and spermatids were very few. Sertoli cells were sometimes dislocated from the basal lamina. Several giant cells were seen in the seminifer tubulus with severe degenerative changes (fig. 3). Oedema was seen in the intertubular areas of the cases with severe degenerative changes. Diameter of those tubules were severely decreased or even completely disappeared in some areas resulting in invasion of connective tissue to these areas and increase of intertubular areas (fig. 4). In the intertubular areas,

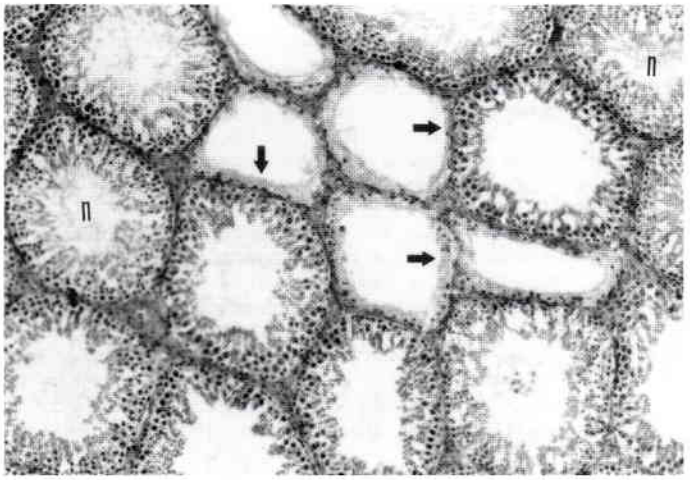

Fig. 1. Testis of a rat fed with 10% CSF on day 60. Note the presence of injured and normal seminiferous tubulus (n). Injured tubulus show germ cell exfoliation and are lined with only a thin layer of germinal epithelium (arrows). H&E × 108

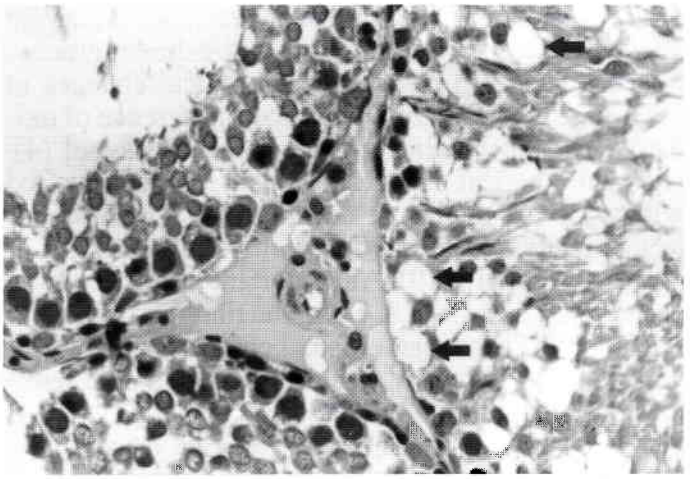

Fig. 2. Testis of a rat fed with 10% CSF on day 40. The marked vacuolization of germinal epithelium in the injured seminiferous tubulus. H&E × 536

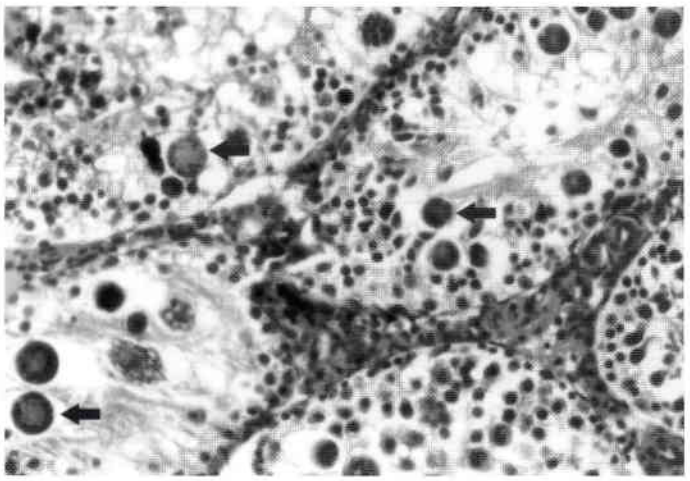

Fig. 3. Testis of a rat fed with 20% CSF on day 80. The marked degeneration and disorganization of germinal epithelial cells, and giant cell formation in the injured seminiferous tubulus. H&E × 268

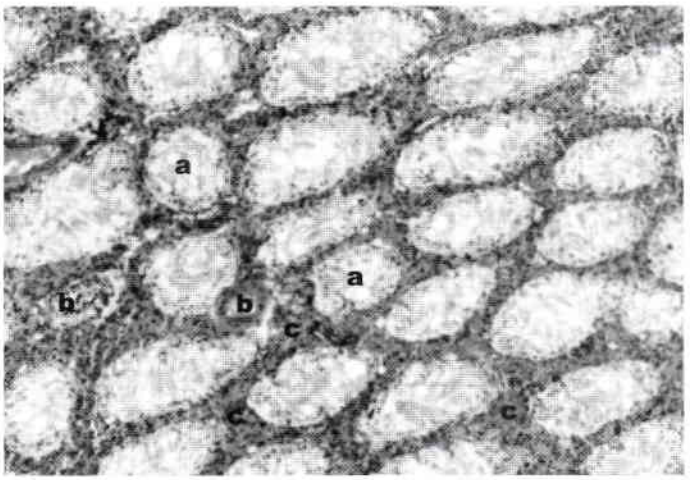

Fig. 4. Testis of a rat fed with 40% CSF on day 60. Severe testicular degeneration, a decrease in diameters of tubules (a), disappearance of some tubules (b), and proliferation of intertubular connective tissue (c). H&E × 108

foamy vacuolation of the Leydig cells was also seen in some areas of sections.

Results of the present study indicated that addition of CSF to the diets of rats at 5% did not affect the sperm motility or did not cause any pathological changes in testicular tissue. On the other hand, CSF addition to the diet at 10%, 20%, 40% decreased the sperm motility at various levels depending on the dose and exposure time and caused microscopic changes in the testicles. In addition, it was an important that clinical symptoms resulted from toxicity was observed only in animals fed with 40% CSF containing diet. However, decrease in sperm motility was found in animals fed with 10% and 20% CSF containing diet, despite the lack of any clinical symptoms.

It has been known that generally, sperm motility > 60% is required for fertility while lower motility rates (< 60%) indicate infertility (4). Similar to the results reported by other researchers on effects of gossypol on sperm motility (2, 8, 9, 16), addition of CSF over 5% to the diets of rats caused a decrease in sperm motility depending on the level and period of exposure. It was noteworthy that sperm motility was completely lost in rats fed with 20% and 40% CSF containing diet on days 80 and 60, respectively. Kalla and Vasudev (7), and Kalla et al. (8) reported that decrease of sperm motility was caused by effects of gossypol on enzymes playing a role in respiratory and energy metabolisms of spermatozoa. Ultrastructural examinations of testicular spermatozoa in gossypol treated rats have revealed a tail lesion described consistently as segmental aplasia of the mitochondrial sheath suggesting that the diminished motility of spermatozoa is probably caused by this damage in the tail (4).

There is a conflict in the literature on the effects of gossypol-acetic acid on weight of testicles. Some researchers reported that gossypol caused a decrease in weight of testicles depending on time and dosage (9, 17) while others reported that gossypol had no effect on weight of testicles (2, 23). In the present study, it

was found that weight and size of testicles of rats were not statistically different among all treatment groups.

It has been reported that pathological changes in germinal cells could be caused by interference of oxidative phosphorilation mechanism by gossypol (4). Histopathological changes determined in the rats fed with > 5% CSF in the present study was found similar to those reported in rats fed with cottonseed (10) or pure gossypol (18, 19). An important difference from previous studies (10, 18, 19) in the findings of the present study was that pathological changes in some animals in group IV and V was determined in both seminifer tubules and intertubular areas.

It was reported that infertility caused by gossypol was reversible by the time despite the fact that sperm motility was decreased dramatically and degenerative changes occured in seminifer tubules (6, 12, 15, 24). Although the present study was not designed to elaborate the question whether the infertility caused by CSF is reversible or not, disappearance of some seminifer tubules in rats fed with 20% or 40% CSF containing diet and proliferation of connective tissue in these areas rise some doubts about that the infertility is reversible.

In this study, in cases with severe tubular degeneration, a number of giant cells were present in the lumen of seminifer tubules. Presence of giant cells was also reported in laboratory animals fed with cottonseed (10) or with pure gossypol (5, 19). However, some researchers reported that giant cells became present when ductus efferent was ligated indicating that this finding may not be an indication of gossypol intoxication but of testicular degeneration (18).

Some researchers (3) published results supporting that gossypol affect Leydig cells while most researchers not (5, 13, 14, 17), indicating a discrepancy on this finding. In the present study, vacuolization in some Leydig cells was seen in cases with severe degeneration of tubules and connective tissue proliferation. This observation may support that Leydig cells may be affected by gossypol when severe degeneration in testicular tissue develops.

It should be noted that the current study was not designed to elucidate the mode of action of gossypol on testicular tissue. It was concluded from the results of the present study that addition of CSF to the diets of rats more than 5% decrease sperm motility and cause pathological changes in testicular tissue. In addition, clinical symptoms may not be necessarily seen in those rats with affected fertility.

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VAESSEN M. M. A. R., KOOISTRA H. S., MOL J. A., RIJNBERK A.: Stosunek kortykoid: kreatynina w moczu u zdrowych psów po doustnym stosowaniu testów supresyjnych z małymi dawkami deksametazonu. (Urinary corticoid: creatinine ratios in healthy pet dogs after oral low-dose dexamethasone suppression tests). Vet. Rec. 155, 518-521, 2004 (17)

Na 11 zdrowych psach w wieku 2-10 lat o masie ciała 7,7-62 kg przeprowadzono badania w celu określenia odpowiedniej dawki deksametazonu zastosowanego w teście 0-LDDST (doustny test supresji przy użyciu deksametazonu stosowany do wykrywania wzmożonego wydzielania hormonu kory nadnerczy). Mocz pobierano do badań o godz. 8.00 i 22.00. Deksametazon w dawce 0,02 mg/kg, 0,01 mg/kg i 0,0075 mg/kg podano z pokarmem po porannym pobraniu próbek moczu. Poziom kortykoidu i kreatyniny oznaczano o 8.00, 12.00, 16.00 i 20.00. Deksametazon w dawce 0,02 i 0,01 mg/kg obniżał stosunek kortykoid: kreatynina oznaczony o 16.00 o ponad 50% w porównaniu do kontroli, w której nie zastosowano deksametazonu. Stosunek ten wynosił poniżej $1{,}0\times10^{-6}$. Natomiast dawka 0,0075 mg deksametazonu/kg obniżała ten stosunek poniżej 50%.

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