



Effects Of Short And Long-Term Administration Of Alfalfa On Testicular Histomorphometry In Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author GM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MH and NE managed the analyses of the study. Author RF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Alfalfa (*Medicago sativa*) is a plant with phytoestrogenic properties, which has been used as a major part of diets in husbandry. Since there are controversial reports related to the effects of alfalfa consumption on animal fertility, its effects on rat testicular tissue were assessed in the present study. Control (n=15) and alfalfa (n=15) groups were fed with ordinary rat chow and ordinary rat chow plus alfalfa, respectively. Testicles were removed after 30, 45, and 60 days of consumption, and tissue sections were prepared to assess histomorphometric changes related to alfalfa consumption.

Based on the results, there was no significant difference in length, width, and volume of testes of treated rats to control in all groups. But the number of testicular spermatogonia cells, primary spermatocyte cells, primary spermatid cells, testicular spermatozoid cells and Leydig cells significantly or insignificantly increased in rats that received alfalfa for 30 days but all of these

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parameters insignificantly decreased in rats that received alfalfa for 60 days. The cause of these changes may be due to estrogenic or anti-estrogenic, antioxidant and endocrine effects of alfalfa.

Conclusion: Consumption of alfalfa for short time had only a transient positive effects on testicular tissues but use of alfalfa for 60 days had little destructive effects on testicular tissue in rats. So longer durations of time could be suggested for further research on the effects of alfalfa on rat's reproduction index.

Keywords: Alfalfa; testes; phytoestrogen; reproduction.

1. INTRODUCTION

Global consumption of estrogenic plants such as legumes is on the rise. Therefore, their probable effects on humans and animals are expanding. Many plants have phytoestrogens and consumption of phytoestrogens may lead to both estrogenic and anti-estrogenic effects and they have varied effects on fertility, such as increased secretions of the reproductive system, infertility, and discontinued sexual behaviors. Differences in the effects of phytoestrogens on reproduction depend on the type and species of plants and the amount of phytoestrogens consumption [1].

Alfalfa (*Medicago sativa*) is a phytoestrogenic plant, which is widely used in traditional and industrial dairy cattle farms. It is one of the most important plants in animal husbandry with phytoestrogens and antioxidative properties. The most important estrogens in alfalfa are Epigenin, Coumestrol, and Coumarin [2-4].

Phytoestrogens are structurally similar to 17 beta-estradiol, in a way that they can attach to the estrogenic receptors and produce biological effects [5].

Both reproductive stimulatory and inhibitory effects have been related to phytoestrogens. It is reported that phytoestrogens accelerate puberty and growth of the genital tract in rodents [6], and alfalfa seed stimulates Leydig cells activity, which may increase blood testosterone levels [7]. On the other hand, Wisniewski et al. (2003) and Weber et al (2001) announced that alfalfa decreased the size of the prostate and sexual organs in male rodents. In addition, phytoestrogenic compounds have been found to cause temporary infertility in domestic female animals [8], an increase in the diameter of the seminiferous tubules, as well as the rate of apoptosis of spermatocytes and spermatids in rats [3].

Experiments have shown that the consumption of phytoestrogens and their effects have increased both in animals and humans [9]. Phytoestrogens

are found in a variety of plants and fodder, and can have adverse effects mainly on the reproductive tract in most animal species, and their effects can vary from an estrogenic over-response, which results in increased secretions in the reproductive tract, to infertility, and disrupting animal behavior. Therefore, many phytoestrogens are now recognized as endocrine disruptor compounds, capable of interfering with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body, which are responsible for reproduction [10].

Interest in the effects of phytoestrogens on male fertility has increased in recent years as it has been demonstrated that estrogens play an important role in the male reproductive system [11]. Although there are only few reports, it has been shown that phytoestrogens also cause reproductive disruption in males [10]. So the aim of the present study was to evaluate the effects of alfalfa, as one of the major sources of phytoestrogens in domestic animals' food on the histomorphometry of rat testis.

2. MATERIALS AND METHODS

2.1 Animals

Male adult Wistar rats (with age: 15-16 weeks with average weight 200-220 g) were provided from house of experimental animals of veterinary medicine of Shahid Chamran University of Ahvaz. The animals were kept at 22±1°C and 12 h light/12 h dark during the one week acclimatization period and during the study. Both groups had free access to tap water. Control group (n=15) had also ad libitum access to rat chow (barley 24.5%, corn 11%, wheat bran 30%, cottonseed meal 4.5%, beet pulp 8%, soybean 10%, beet molasses 1.5%, vitamin premix 7%, calcium carbonate 1.5%, salt 2%), while the access of the alfalfa group (n=15) to food had been limited to force them to eat alfalfa. Thus, the latter group were fed daily with 80 g rat chow plus at least 300 g (after experiments the amount

of intake was achieved) fresh alfalfa (provided from farm of Agriculture faculty of Shahid Chamran University of Ahvaz). At days 30, 45, and 60, left testis of five rats from each group were collected to undergo histomorphometric studies.

2.2 Sample Collection

Five rats from control group plus five rats from alfalfa group were sampled at days 30, 45, and 60 following alfalfa consumption. Animals were anesthetized with ketamine-xylazine mixture (75 mg/kg – 5 mg/kg) and euthanized. Left testis and epididymis were removed. Left testis dimensions and weight were measured, and immediately transferred to a 10 percent formalin solution. Paraffin-embedded tissue sections (5-6 um) were stained with Hematoxylin-Eosin to assess

spermatogenic layer, Sertoli and Leydig. Digital Lens and Dino capture 1 software were used for counting and measuring.

2.3 Statistics

The data were analyzed using SPSS 16 software by independent T student test ($P < 0.05$).

3. RESULTS

According to the Table 1, which shows the changes in the testicular dimensions, volumes, and weights in different time durations, the diameter and weight of the testes have been increased following 60 days of alfalfa supplementation compared to the control group ($p < 0.05$).

Table 1. Mean (\pm SD) of the dimensions, volume, and weight of the testicles at days 30, 45, and 60 following alfalfa supplementation

	Day 30		Day 45		Day 60	
	Control	Alfalfa	Control	Alfalfa	Control	Alfalfa
Length (mm)	17.6 \pm 0.89	18.2 \pm 1.09	17.4 \pm 0.54	18.6 \pm 0.89	17.5 \pm 1.41	18.6 \pm 0.89
Width (mm)	9.7 \pm 0.44	9.8 \pm 0.48	9.2 \pm 0.44	9.6 \pm 0.55	9.00 \pm 0.83	9.7 \pm 0.44
Diameter (mm)	34.2 \pm 1.78	34.6 \pm 0.54	34 \pm 1.22	35 \pm 0.7	34.8 \pm 1.3	36.8 \pm 0.44*
Volume (mm ³)	1.32 \pm 0.1	1.4 \pm 0.14	1.32 \pm 0.13	1.4 \pm 0.13	1.28 \pm 0.22	1.42 \pm 0.1
Weight (g)	1.37 \pm 0.1	1.42 \pm 0.13	1.4 \pm 0.13	1.5 \pm 0.1	1.43 \pm 0.22	1.7 \pm 0.09*

*Significant level of $P < 0.05$

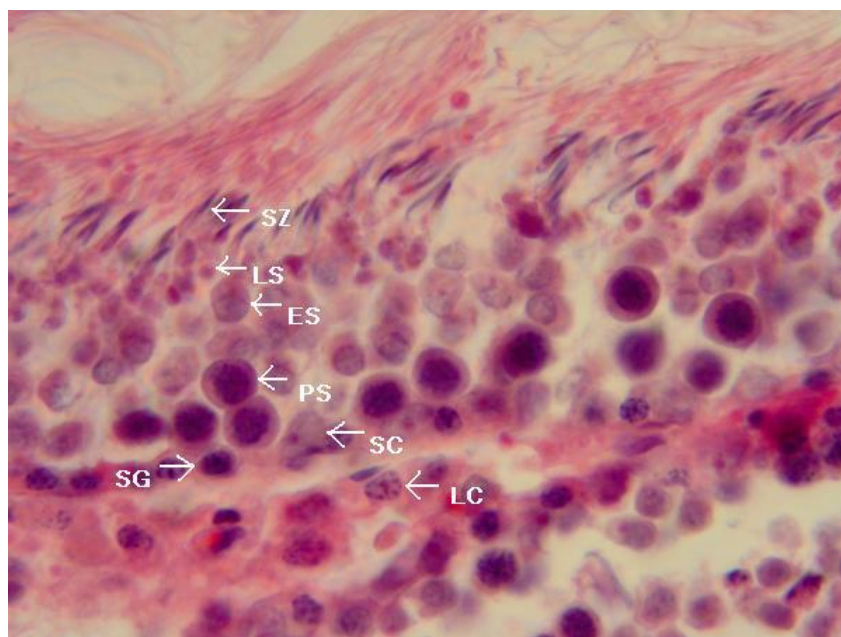


Fig. 1. Types of spermatogenic cells in the first control group. SG (spermatogonia), SC (sertoli cell), PS (primary spermatocyte), ES (early spermatid), LS (Late spermatid), SZ (spermatozoid), LC (leydig cell) (H&E.100 \times)

As could be seen in Table 2 no significant differences in the number of seminiferous tubules, wall thickness and diameter of seminiferous tubules, numbers of Sertoli cells, Spermatogonia, primary and secondary Spermatids, and Leydig cells between control

and alfalfa groups at days 30, 45, and 60 were observed. However, the number of primary Spermatocytes and the number of testicular spermatozoid cells were higher in alfalfa group at day 30.

Table 2. Mean number and diameter of seminiferous tubules, sertoliceLls, spermatogonia, spermatocyte, spermatid, and leydig cells

Group (mean)	Day 30		Day 45		Day 60	
	Control	Alfalfa	Control	Alfalfa	Control	Alfalfa
Seminiferous tubules (n)	20.78±3	20.96±2.45	24.62±2.52	28.48±3.25	22.94±2.76	20.64±1.82
Wall thickness of seminiferous tubules (µm)	63.29±3.10	65.96±3.77	54.98±2.59	56.66±2.66	53.23±2.69	62.65±1.28
Seminiferous tubules diameter (µm)	20.07±2.54	20/1±2.45	24.86±2.52	28.24±2.25	22.89±2.81	2.86±1.82
Sertoli cells (n)	3.12±0.61	2.69±0.31	2.8±0.52	2.48±0.28	2.8±0.54	2.64±0.44
Spermatogonia (n)	7.59±1,67	9.13±1.33	10.1±1.84	10.77±1.11	8.76±1.97	10.46±1.29
Primary spermatocytes (n)	9.67±1.6	11.86±1.1*	12.63±1.31	14.22±1.59	12.3±0.61	11.96±0.88
Primary spermatids (n)	17.96±1.84	17.37±1.29	18.58±1.54	18.53±1.19	18.06±1.04	17.98±1.4
Secondary spermatids (n)	10.64±1.4	9.12±2.78	9.82±1.86	8.46±2.2	10.3±2.6	11.3±2.1
Spermatozoids (n)	11.3±1.2	14.94±1.94**	13.64±1.4	14.16±1.54	14.25±1.6	14.16±1.73
Leydig cells (n)	16.3±1.42	17.14±1.63	17.72±1.2	17.11±1.72	16.12±1.3	15.8±1.8

*Significant level of $P < 0.05$

**Significant level of $P < 0.01$

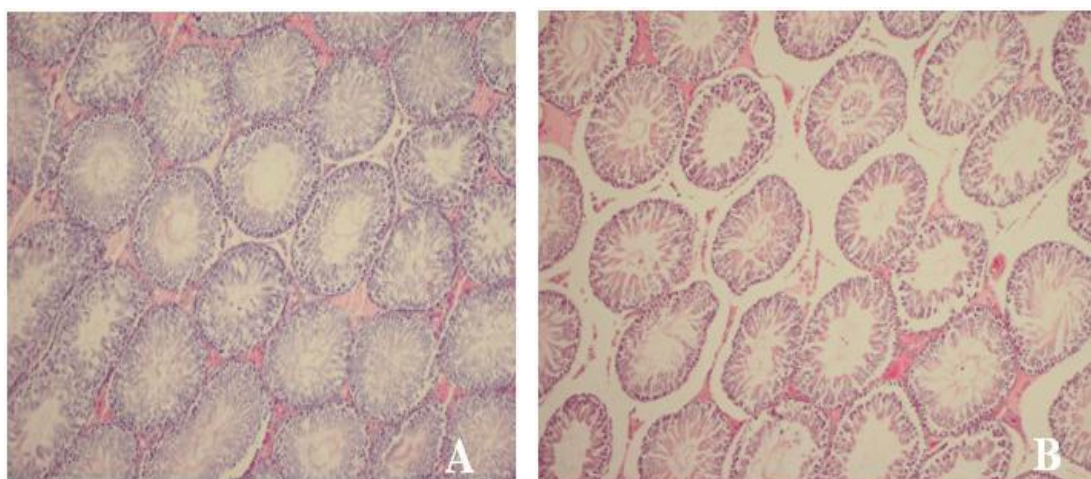


Fig. 2. The same dispersion of seminiferous tubules in the first treatment (B) and control groups (A) (H&E.10×)

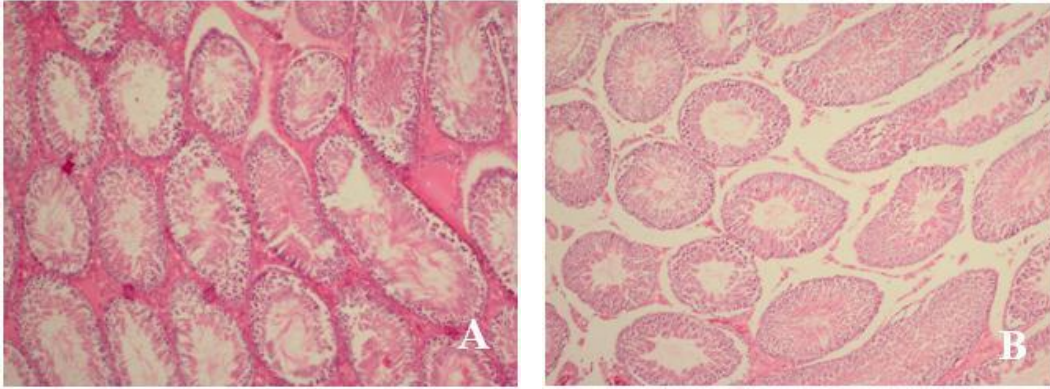


Fig. 3. No significant changes in diameter of the seminiferous tubule of third treatment (B) compared to the control groups (A) (H&E.10×)

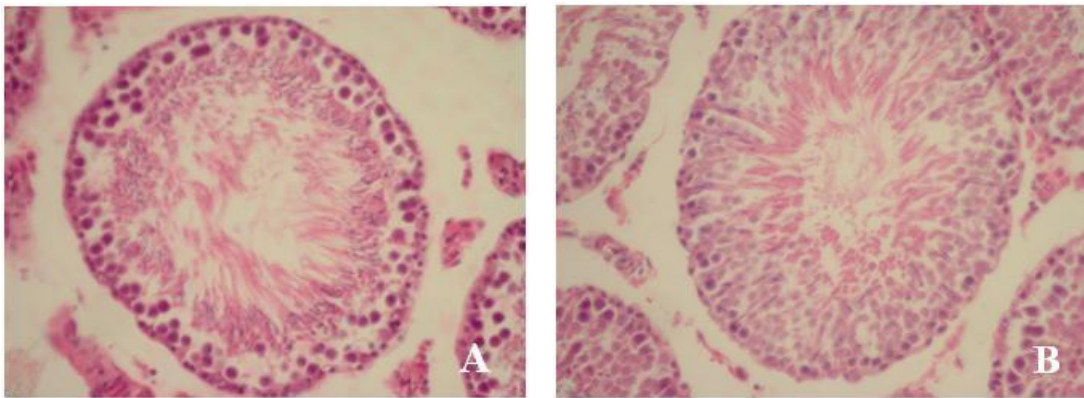


Fig. 4. The insignificant increase in the thickness of the wall of the seminiferous tubules of third treatment (B) compared to the control groups (A) (H&E.40×)

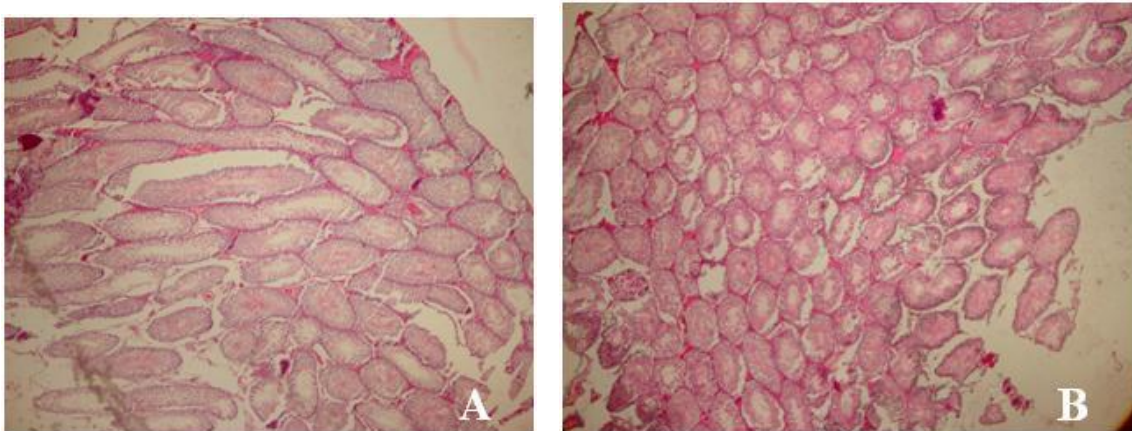


Fig. 5. The insignificant increase in the number of seminiferous tubules in second treatment (B) compared to the control groups (A) (H&E.4×)

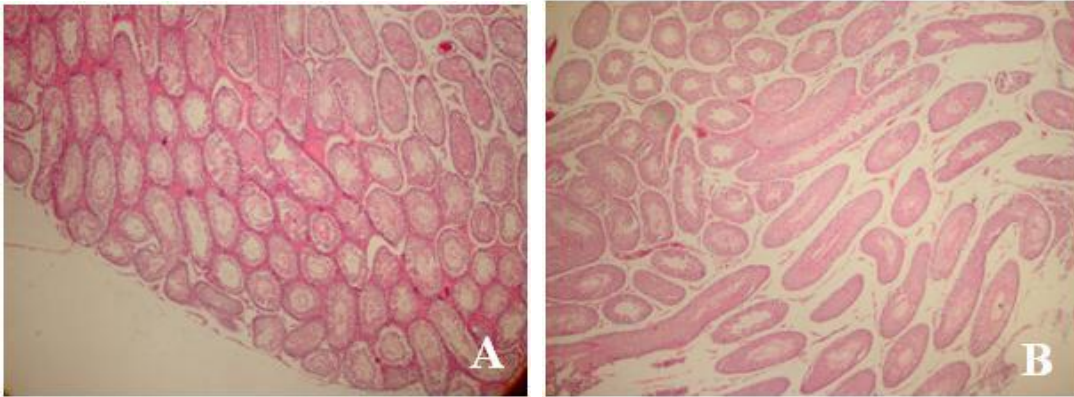


Fig. 6. The insignificant reduction in the number of seminiferous tubules of third treatment compared to the control group (A) (H&E.4×)

4. DISCUSSION

Based on the data of the present study the diameter and weight of the testes were significantly increased following 60 days of alfalfa consumption in comparison to the control group and rats that received alfalfa for 30 days. The difference between effects of phytoestrogen on testicular weight and volume can be due to the difference in the duration and rate of consumption and type of phytoestrogens [12]. For example consumption of leaf extract of *Sesamum radiatum* for six weeks could increase weight of testes in rats [13]; on other hand long time consumption of a soy diet high in isoflavone/polyphenolic molecules increase testes weight in young adult male rats in comparison to those that were provided with the same for short time [14]. This may be due to reflection of the site of action of the sesame phytoestrogens, which tend to bind to estrogen receptors (a and b) present in the testes [13].

Based on the results, consumption of alfalfa had only a transient effect on the number of seminiferous tubules, primary Spermatocytes and Spermatozoids. Since the phytoestrogens have both estrogenic effects and anti-estrogenic effects [15] Therefore, increase in the number of seminiferous tubules, spermatocyte and spermatozoid may be due to the estrogenic effects of alfalfa.

The number of testicular spermatogonia cells, primary spermatocyte cells, primary spermatid cells, testicular spermatozoid cells and Leydig cells significantly or insignificantly increased in rats that received alfalfa for 30 days but all of these parameters insignificantly decreased in

rats that received alfalfa for 60 days. The cause of these changes may be due to estrogenic or anti-estrogenic [10,15], antioxidant [3] and endocrine effects of alfalfa [16].

The differences between rats that consumed alfalfa for 30 and 60 days can be due to the amount of consumption or long use of alfalfa [10] because long consumption of phytoestrogen can reduce fertility in male and female [2,3].

Alfalfa can change testosterone, estradiol, LH and FSH in rats [16] therefore the changes of parameters in present study may be due to changes of reproductive hormones. On other hand alfalfa has antioxidant effects [1] so may be this effect can change fertility of rats too; because these agents can directly or indirectly reduce spermatogenesis [17-19].

In the present study, there was insignificant increase in the number of Leydig cells in first treatment group and insignificant decrease in second and third treatment groups. This effect may be due to effects of phytoestrogens on Leydig cells as phytoestrogen can reduce the proliferation of Leydig precursor cells [20-22]. The decline of Leydig cells may be due to phagocyte by aggressive macrophages [23]. The difference between first and third treatment groups of the present study may be due to duration of consumption of alfalfa.

5. CONCLUSION

In conclusion, this study showed that the consumption of alfalfa for 30 days had transient positive effects on testicular tissues in rats but for

60 days had little destructive effects on testicular tissues effects. However, it suggests the use of alfalfa for longer duration in rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the authors. (Ethical permission code: EE/97.24.3.49896/scu.ac.ir).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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