

## Effect of Diet Contains Sesame Seed on Adult Wistar Rat Testis

Efecto de la Dieta con Semillas de Sésamo sobre el Testículo de la Rata Wistar Adulta

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**SUMMARY:** Studies show that some antioxidants are effective in improving male infertility. According to several antioxidant compounds that exist in sesame seed, this study was designed and carried out to the effects of sesame seed diet consumption on adult male rats testis structure and sex hormones. This experimental study was carried out on 30 adults Wistar rat, 200 g that obtained from laboratory animal center at Kashan University of Medical Sciences. Rats were divided into experimental and control groups randomly. The control group received standard diet and experimental group received diet containing 70% standard diet and 30% sesame seed after weaning for 12 weeks. At the end of the study, testis weight and volume were measured and seminiferous tubules; lumen epithelium diameter, LH, FSH and testosterone concentrations were evaluated. Data was analyzed by SPSS software and t-test.  $P < 0.05$  was considered to significant level. Bodyweight rats, weight and volume testis and percentage volume seminiferous tubules vessels in two groups were not significant. The mean cells number and motility of sperm in left epididym, number of cells epithelium and percentage volume of epithelial, lumen and interstitial of this tubules were extremely significant ( $P < 0.0001$ ) in the experimental group compared to control. LH concentration increased significantly in the experimental group compared to control ( $P < 0.03$ ). Sesame seed intake improved testicular parameters, fertility and sperm production in males.

**KEY WORDS:** Sesame seed; Testis; Rat; Sex hormones.

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### INTRODUCTION

Infertility is one of the medical problems in the world so that about 15-10 percent of couples have experienced some form of infertility problem (Kamali *et al.*, 2006). It has been reported that 30% of couples infertility problems related to male, 40% to 50% related to female and 20% to 30% related to both sexes (Esmaeilzadeh *et al.*, 2002). In recent years many efforts have been made to identify an ideal herb with strong and effective anti-metabolic effects on male fertility which already has created a serious problem. Phytoestrogens affect the reproductive system of male animals which have attracted the attention of researchers. Phytoestrogens are phenolic compounds which are similar to hormones and nonsteroidal that derived from estrogen of sesame and is found in sesame seed (Shittu, 2006).

Sesame (*Sesamum indicum*) is one year old seed and belongs to the Pedaliaceae family (Zavareh *et al.*, 2008). Sesame is one of the richest dietary sources of lignan, Phytoestrogens which exist in it were known to human from

the beginning of civilization and they are mixed with human food because of having many benefits for health (Thompson *et al.*, 1991). Sesame lignan, such as: Sesamin, Sesamol, Sesaminol, Sesamolol, Pinorsinol, Sesamol and gamma-Tocopherol which are isolated from *Sesamum indicum* and *Sesamum radiatum* seeds and they have more tumorigenic, estrogenic or anti-estrogenic and antioxidant features compare with other plant species (Shittu; Jeng & Hou, 2005; Jayalekshmy *et al.*, 2001). In terms of phytochemical, this plant has phenolic compounds (Phenols, Sterols, Flavonoids and lignans), non-protein amino acids, cyanogenic glucoside, alkaloids, unsaturated fats and lipids with multiple double bonds, glazes, phospholipids and E, B1 and B2 vitamins. Minerals or trace elements such as calcium, iron, magnesium, zinc, copper and phosphorus exist in this plant (Konan *et al.*, 2008). Approximate analysis of sesame seed has made it clear that the seeds contain 50-60% oil, 8% protein, 5.8% water, 3.2% crude fiber, 18% carbohydrate and 5.7% ash (Obiajunwa *et al.*, 2005; Konan

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*et al.*). Ewe atura (one of the local name in southeast Nigeria) means leaves that provide comfort and health for the body because of being useful for treatment of constipation and digestive disorders. It has been reported that boiling a mixture of roots and leaves has antiviral and antifungal activity (Krinke, 2000; Gills, 1992). Shittu *et al.* (2007) who evaluated the effect of fluid extract of sesame leaves on epididymal spermatocyte reserves in adult rats, concluded that sesame leaves increase fertility. Also researchers, who evaluated the effects of leaves extract of this plant on the fertility of hypoglycemic rats, found that this extract improves parameters related to the testis and increases reproductive potential in mice (Shittu *et al.*, 2009). According to the above and lack access to adequate scientific evidence on the effect of sesame seed on testicular parameters, this study investigated the effect of sesame seeds dietary on the testes of adult male rats.

## MATERIAL AND METHOD

**Classification and grouping animals** Thirty healthy adult Wistar rats weighing 200 g were obtained from animal center of Kashan University of Medical Sciences and were kept in wire cages in the animal house of the University. After infancy, they were protected and controlled under the light regime (12 h light: 12 h dark), at room temperature ( $2\pm 22^{\circ}\text{C}$ ) and humidity constant ( $5\pm 55\%$ ). During the study (12 weeks) rats were fed a diet of standard pellets and had free access to water.

**Preparing food.** Normal food powder of rats were collected and sieve and white sesame seeds available in the market, were powdered with domestic mill, then the powder prepared from normal food of rat (70%) with ground sesame seeds (30%) were mixed and turned to a pulp with water and became tubular shaped with the pastry cone then they were put in trays within a period of 2-3 days and then were dried in open air and given to rats.

### Qualitative and quantitative studies

**Body weight.** Rats were weighing every week with a digital scale and weights were recorded. After animals were anesthetized and incision made on the chest and abdomen, first, blood sample was taken from the heart and their testes were removed.

**Weight and testicular volume.** Both testes were dissected carefully and all the fat was removed. Testicular weight was measured with a sensitive scale and their volume was measured with water displacement in a 10 ml cylinder.

**Motility and sperm count in the epididymis.** After anesthetizing, scrotal incision was performed and testis and epididymis were appeared. The left epididymis was dissected and transferred to HAMS F10 culture and with a few incisions in the head, trunk and tail of the epididymis, provides sperm to come out and after 20 min, culture containing microliters of sperm were placed on slides then sperm parameters (count and mobility) were measured.

**Measurement of serum hormones.** FSH and LH concentrations were measured by using the ELISA technique in samples of 50 microliters. Evaluation of serum testosterone was performed by using the Chemo-Luminance.

### Testicular tissue evaluation

**Counting the spermatogenic cells.** For this purpose 4 sections have been studied randomly, and from each section, 5 spermatogenic duct in testes (to increase the accuracy of counting) have been chosen which were in stages VII and VIII of the cell cycle and had a perfectly circular cross section (3). Cell counting was done by using a Zeiss optical microscope with a magnification of 40X. In each duct spermatogonia cells, spermatocyte in pachyten stage, round spermatid and elongated spermatid have been counted and after determining the average cell in a duct of one group, were compared with cells of other group.

**Determining the volume percent.** For this purpose point-counting was used for the volume percent of epithelium, interstitial space and seminiferous tubule lumen. These were measured by using a Zeiss optical microscope with 10X magnification. To determine the volume percent of the components of testicular tissue, 6 sections were used in two perpendicular directions to each other with equal intervals and non-overlapping.

**Measuring the diameter of the seminiferous tubule.** For measuring this parameter, Zeiss optical microscope with Eye Piece micrometer was used which had X10 magnification calibrated by Stage Micrometer. Desired parameters (seminiferous tubule diameter) thirty, seminiferous tubule (6 sections and each section with 5 tube) with circular or nearly circular cross section were measured in each testis (Krinke).

**Statistical analysis.** The data were prepared as Mean $\pm$ SEM and statistical analysis was performed by using student's t-test and one way ANOVA analysis.  $P<0.05$  was considered to be significant.

## RESULTS

This study was done on 30 adult male Wistar rats weighing 190-210 g. The rats were randomly divided into control and experimental groups. Control group used normal diet and experimental group fed 70% normal diet and 30% sesame seed after infancy for 12 weeks. Significant differences were not observed in animal body weight, testicular weight and volume in both control and experimental groups (Table I). Sperm number and mobility in left epididymis significantly increased in experimental group compared with control group (Table I). LH levels significantly increased in experimental group compared to controls but significant changes in FSH and testosterone levels were not observed in both groups (Table I). In This study spermatogonia cells, primary spermatocyte,

spermatid and spermatozoa increased significantly in both right and left testes of the experimental group compared to control group ( $P < 0.0001$ ). Changes in volume percent of epithelium and lumen in both testes significantly increased in experimental groups compared to control group and interstitial space of seminiferous tubules significantly decreased in experimental group compared with the control group ( $P < 0/0001$ ), also in this study, changes of were vascular volume detected non-significant in both groups. According to the results of this study, the diameter of seminiferous tubules with a probability level ( $P < 0/0001$ ) in both right and left testes of the experimental group showed a significant increase compared with the control group (Table I).

Table I. Various parameters in control and experimental groups of male rats.

Variable	Control group (Mean± SEM)	Experimental group (Mean± SEM)	P-value
Body weight (g)	195.88±7.67	194.42±8.04	0.89
Testis weight (g)	0.026±1.45	1.5025±0.029	0.235
Testis volume (cm <sup>3</sup> )	5.672±0.242	5.485±0.244	0.599
Sperm number (x 10 <sup>6</sup> )	62.14±3.91	74.23±2.52	0.017
Sperm motility (%)	57.86±3.77	72.31±2.98	0.006
LH (IU/L)	2.7±0.29	3.5±0.22	0.031
FSH (mIU/L)	3.9±0.44	3.3±0.28	0.27
T (nmol/L)	10.88±2.9	8.45±2.9	0.57
n of Spermatogonia/Tubule	40.49±4.62	50.22±5.18	<0.0001
n of Primary spermatocyte/Tubule	47.7±5.45	59.86±6.46	<0.0001
n of Spermatid/Tubule	114.66±13.82	149.15±17.3	<0.0001
n of Spermatozoa/Tubule	116.28±12.84	146.64±16.12	<0.0001
Volumetric density of the epithelium (%)	48.76±0.515	55.82±0.47	<0.0001
Volumetric density of lumen (%)	13.04±0.26	14.31±0.22	<0.0011>
Volumetric density of Interstitial space (%)	37.7±0.66	29.14±0.51	<0.0001
Volumetric density of vessels (%)	0.615±0.14	0.795±0.11	0.337
Tubular diameter (µm)	210.62±1.62	248.42±1.82	<0.0001

Results expressed in mean ± S.E.M (Standard Error of Mean). n=15.

## DISCUSSION

In this study, the number of spermatogonia cells, primary spermatocyte, spermatid and spermatozoa increased significantly in both right and left testes of the experimental group compared to control group ( $P < 0.0001$ ). Statistical increase in cells can be attributed to mitosis and meiosis

division and factors affecting cytokinesis (Berne & Levy, 1993). The results of this study are in accordance with the study of Shittu *et al.*, (2008) who found that the extract of sesame leaves, increases the number of spermatogonia cells of epithelium seminiferous tubules in the experimental group

compared to control, this high number is due to increased primary spermatocyte which is the result of increasing proliferation of stem cells or increasing in spermatogenesis due to large mass of epithelial in these tubules. Shittu *et al.* (2009) concluded that the number of cells in seminiferous tubules significantly increased in diabetic mice compared with control group which are consistent with the results of this study but these researchers examined the effect of fluid extract of sesame leaves on diabetic rats whereas the sesame seeds were used in this study.

According to the results obtained in this study, the volume percent of epithelium and lumen significantly increased in testes of experimental group compared with control group ( $P < 0.0001$ ) and interstitial space of seminiferous tubules significantly decreased in the experimental group compared with the control group ( $P < 0.0001$ ) but in this study, vascular volume percent was detected non-significant in both groups. Study of Shittu *et al.*, (2009) showed that in hypoglycemic rats which were receiving fluid extract of sesame leaves, the average lumen volume percent were increased and interstitial cells volume percent of seminiferous tubules of testis were decreased (Huang *et al.*, 1987) which is consistent with results of this study but it is noteworthy that these researchers studied the sesame leaves extract dietary on rats. It is expected that sesame will stimulate spermatogenesis mechanisms via such as epithelial proliferation and increased lumen and tubular thickness, especially from stages V to VII of spermatogenesis (Shittu *et al.*, 2009).

According to the results of this study, highly significant changes were observed in diameter of seminiferous tubules with a probability level ( $P < 0/0001$ ) in the testes in the experimental group compared with control group. FSH level is the main factor in the growth of seminiferous tubules and testicular size, also Ribnicky *et al.*, (2006) and Shittu *et al.* (2008) who studied on the effect of sesame leaves on rats, found that FSH and testosterone are involved as synergistic in the process spermatogenesis (Berne & Levy) which is inconsistent with our results because the concentrations of these two hormones in this study did not show significant changes, in addition normal testicular growth and development requires the transcription of DNA molecules and proteins function in the testes. Changing the diameter of these tubules may be for two reasons: A) Increased androgens, increase protein synthesis of seminiferous tubules (Hadley, 1996). B) Increased number of stem cells of seminiferous tubules cause a change in diameter. According to this results, Shittu *et al.*, (2009) showed that consumption of fluid extract of sesame leaves increased the mean diameter of seminiferous tubules in the experimental group (consumer extract) compared to the control group (not taking the extract) ( $P < 0/05$ ).

Sesame is very useful for humans due to the economic and medicinal value. These seeds are rich as minerals and trace minerals; vitamins and antioxidant lignan (phytoestrogens) and can improve the fertility potential of male reproductive tract (Shittu *et al.*, 2008). We know that the beneficial effects of high intake of fruits and vegetables that just do not affect different conditions of body's metabolic disease (Such as diabetes mellitus, obesity, heart disease and cancer) but also has positive effects on person reproductive tract (Shittu *et al.*, 2008). Many antioxidant or non- antioxidant phytochemical compounds with additive or synergistic activities of various compounds found in fruits such as alpha-linolenic, various phenolic compounds (sesamol, sesamin) and fibers exist in sesame seed. Thus, it seems that estrogen receptor-alpha is useful in regulating reproductive physiology estrogen, including components of behavior compared to the estrogen receptor beta (Zaneveld & Polakoski, 1977).

Significant increase was observed in LH levels in the experimental group compared to controls ( $P < 0.03$ ). But significant changes in FSH and testosterone levels were not observed in both groups. Shittu *et al.*, (2008) who studied the sesame leaves extract on rats found that sesame phytoestrogen lignans can stimulate testosterone aromatization to estradiol, or convert to dihydrotestosterone, so it can be concluded that decreasing the concentrations of testosterone, is because of converting it to estradiol which is done by aromatase and reductase enzyme and sesame lignans. Huang *et al.*, concluded that less than 25% concentration of testicular testosterone is sufficient to protect all stages spermatogenesis.

According to the study of Shittu *et al.* (2008), FSH concentration decreased in consumer of high dose liquid extract of sesame leaves group compared with control group and it does not match to our results, the result differences obtained from these studies with our results are in the type of diet (extract of sesame leaves), type of rats and duration of using diet. However, FSH has a synergistic effect with testosterone hormone and stimulating synthesis of the androgen receptor at the receptor level. Plant & Marshall (2001) found that treating the removed pituitary rats with FSH stimulates spermatogenesis with androgen binding protein (ABP) in testis and epididymis.

In the present study, sperm number in the left epididymis significantly increased in experimental group compared to the control group ( $P < 0.0172$ ), also sperm mobility in the left epididymis was significantly higher in experimental group compared to the the control group ( $P < 0.0064$ ). Obtained results were consistent with the study of Shittu *et al.*, (2008) who obtained the number of cells and spermatozoa from the caudate of epididymis with Zanol and Pockashi method (Zavareh *et al.*), the researchers found that the number

of sperm in the caudate of epididymis significantly increased, this increase may be due to increased spermatocyte which is the result of increased stem cell proliferation or increase in spermyogenesis (Shittu *et al.*, 2008). Also Shittu *et al.*, (2009) found that liquid extract of sesame leaves, increases sperm mobility, which may be due to antioxidant properties of sesame on free radicals that are likely consistent with the our results.

Experimental rats and the control group did not show any significant increase in body weight which was similar to the study of Awoniyi *et al.*, (1997) who evaluated the effect of phytoestrogens on spermatogenic potential in rats. According to the study of Shittu *et al.*, (2009, 2007) who suggested that the fluid extract of sesame leaves significantly increase the weight of rats, which was inconsistent with the results of this study. The sesame seed is used in this study, and giving this diet for a long time may increase body weight.

Weight and volume of testis showed no significant changes in all groups. Ashamu *et al.*, (2010) studied the ethanolic effect of sesame seed extract and vitamin C on fertility of Wistar rats, concluded that significant differences were observed in testicular weight in the vitamin C consumer group (as one of the antioxidants in sesame seed) compare with control group but the group which used ethanolic extract of sesame extract alone and received it with vitamin C simultaneously, testicular weight was more in comparison with control group. The increase in testicular weight can be attributed to a combination of high fat and calories of seeds, which researchers showed that using vitamin C alone had no effect on testicular weight and volume. It is worth noting that the researchers studied the ethanolic effect of sesame extract and vitamin C on the parameters associated with testis. Kuiper *et al.*, (1997) who evaluated the differences between the ligand binding to estrogen receptors alpha and beta in the testis, found that testis weight loss in experimental group comparison with the control group is due to its estrogenic action of sesame lignin which is depending on estrogen receptors binding (alpha and beta) in the testis compare with receptor in epididymis which is inconsistent with the results of our study. Thus, estrogen or its receptor is important for normal functioning of the reproductive system of different species. Whereas the study of Shittu *et al.*, (2009) showed that weight and size of testis in rats which have used the fluid extract of sesame leaves, had significant differences compared with control groups. It seems that the cause of differences in our results compared with the results of study of Shittu *et al.*, (2007, 2009) may be the use of liquid extract of sesame leaves, whereas we used sesame seed in this study, also, the rat race in these two studies were different. Oral administration of seed for long-term may cause significant changes in testicular weight and volume, also it is likely that significant changes will observed by increasing the number of tested animals.

## CONCLUSION

This study shows that sesame seed increase LH and improves spermatogenesis process, also, the seed oil has no effect on body weight.

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MAHABADI, J. A.; BAFRANI, H. H. & NIKZAD, H.; TABERIAN, A. & SALEHI, M. Efecto de la dieta con semillas de sésamo sobre el testículo de la rata Wistar adulta. *Int. J. Morphol.*, 31(1):197-202, 2013.

**RESUMEN:** Estudios demuestran que algunos antioxidantes son eficaces en la mejora de la infertilidad masculina. Debido a la presencia de varios compuestos antioxidantes que existen en la semilla de sésamo, este estudio fue diseñado y realizado para evaluar los efectos de su consumo sobre la estructura testicular y las hormonas sexuales de ratas macho adultas. Se utilizaron 30 ratas Wistar adultas de 200 g obtenidas desde el centro laboratorio animal de Kashan University of Medical Sciences. Las ratas se dividieron en grupos experimental y de control. El grupo control recibió una dieta estándar y el grupo experimental una dieta que contenía 70% de dieta estándar y 30% de semillas de sésamo, después del destete durante 12 semanas. Al final del estudio, se midieron el peso y volumen de los testículos y túbulos seminíferos, diámetro luminal epitelial, y las concentraciones de LH, FSH y testosterona. Los datos fueron analizados mediante t-test con el programa SPSS. Fue considerado significativo un valor  $P < 0,05$ . El peso corporal de las ratas, peso y volumen testicular, y el porcentaje volumétrico de los túbulos seminíferos en los dos grupos no fue significativo. La media del número de células y la motilidad de los espermatozoides en epidídimo izquierdo, número de células del epitelio y porcentaje volumétrico del epitelio, y lumen intersticial de los túbulos fueron significativos ( $P < 0,0001$ ) en el grupo experimental en comparación con el control. La concentración de LH aumentó significativamente en el grupo experimental en comparación con el control ( $P < 0,03$ ). La ingesta de semillas de sésamo mejora de los parámetros testiculares, la fertilidad y la producción de espermatozoides en machos.

**PALABRAS CLAVE:** Semillas de sésamo; Testículo; Rata; Hormonas sexuales.

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