

Effect of Diet Contains Sesame Seed on Epididymal Histology of Adult Rat

Efecto de la Dieta con Semillas de Sésamo sobre
la Histología del Epidídimo de la Rata Adulta

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SUMMARY: Sesamin is a major lignan constituent of sesame seed and considered as a key factor in a number of beneficial effects on human health. Sesame leaves intake improve and increase epididymal spermatocytes reserve in adult male sprague dawley rat. The present study was aimed to investigate the effects of contain sesame seed on epididymis histological structure. Thirty adult male rats were divided into two groups of 15 rats each. The regimen group received diet containing 30% sesame seed, while the control group received diet along 12 weeks. The right epididymis were removed and minced into several pieces on a specimen bottle containing normal saline for some few minutes to allow the sperms to become motile and swim out and sperm parameters were analyzed. Left epididymis were divided into three sections and fixed into bouin,s solution for further investigation. Serum FSH and LH concentration were estimated by ELISA technique, Testosterone concentration was done by using Chemo-Luminance method. The body weight gained during the treatment period did not differ significantly among groups. The mean epididymal sperm motility and count of the experimental group was significantly higher than control group. LH levels significantly increased in experimental group compared to controls but significant changes in FSH and testosterone levels were not observed in both groups. The results obtained showed that the mean epididymal diameter of the tubular, lumen and epithelium in three parts were not significant in two groups. It can be concluded that sesame seed improves sperm parameters (motility and count) and also can increase LH. But diet contains sesame seed did not affect on epididymal tissue and body weight.

KEY WORDS: Diet; Epididymis; Sesame seed; Sperm; Hormone.

INTRODUCTION

The function of the epididymis, including production of the epididymal specific microenvironment necessary for the maturation, storage, and survival of spermatozoa, is regulated by hormones and testicular growth factors (Swider-Al-Amawi *et al.*, 2010). More recently, it has been hypothesized that both testicular cancers and sub-fertility may be caused by the exposure of the developing male embryo to agents that disrupt normal hormonal balance (Sharpe & Skakkebaek, 1993; Sharpe, 2003; Izegbu *et al.*, 2005).

Sesame (*Sesamum indicum*) is an important oil seed crop due to its high nutritional and therapeutic values and cultivated widely in tropical, subtropical, and southern temperate regions (Chakraborty *et al.*, 2008). Sesamin is a major lignan constituent of sesame seed and considered as a key factor in a number of beneficial effects on human health.

These benefits include anticancer properties (Yokota *et al.*, 2007), antihypertensive properties (Miyawaki *et al.*, 2009), anti-inflammatory properties (Jeng *et al.*, 2005), antioxidative properties (Nakano *et al.*, 2003), cholesterol lowering activity (Chen *et al.*, 2005), enhancement of hepatic fatty acid oxidation and alcohol metabolism (Tsuruoka *et al.*, 2005), neuroprotection (Khan *et al.*, 2010) and promotion of angiogenesis (Chung *et al.*, 2010).

In addition, sesame plant is one of the richest food sources of phytoestrogenic lignans, a valuable phytochemical known to man since the dawn of civilization (Thompson *et al.*, 1991) and is now increasingly being incorporated into human diet worldwide because of their reported health benefits (Shittu *et al.*, 2009a). Shittu *et al.* (2007) reported that sesame leaves intake improve and increase epididymal spermatocytes reserve in adult male sprague dawley rat.

According to results Shittu *et al.*, (2007) that they studied improved fertility potential and antimicrobial activities of sesame leaves phytochemicals, the mean epididymal diameter and volume density of the tubular lumen significantly increased by respectively in low dose sesame as compared to the control group. In addition, Sesame leaves extract consumption enhances the quality of the spermatozoa produced with improvement in the storage capacity of the epididymis for these spermatozoa in a dose related manner (Shittu & Shittu, 2012).

Scientists studied on influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels', found that zinc (as a element sesame seed) (Obiajunwa *et al.*, 2005) supplementation to the lead-exposed rats revealed the protective effect of zinc on these parameters. Zinc could compete significantly and effectively reduce the availability of lead binding sites (Batra *et al.*, 2001).

The aim was to carry out the effect of diet contains sesame seed on epididymis histological adult wistar rat epididymis using histomorphometric methods and hormonal assay.

MATERIAL AND METHOD

Animal experiment. Thirty mature and healthy adult male wistar rats weighing 190 to 210 g were procured from kashan University, College of Medicine, kashan-iran from 2010-2011. They were housed in well ventilated wirewooden cages in the departmental animal house. They were maintained under controlled light schedule (12 h light and 12 h darkness) at room temperature (28°C) and with constant humidity (40 - 50%). The animals were allowed to acclimatize for a period of 7 days before treatment during the experiments. During this period (12 weeks period) they were fed with standard rat chows/pellets supplied and water *ad-libitum* (Shittu *et al.*, 2007; Shittu *et al.*, 2008).

Experimental design. An experimental study was designed in that two types of rats (A and B) were randomly selected and were assigned into experimental and control groups. The group A served as the control while B constituted the treated group. The group A animals received normal diet while group B received normal diet 70% + seeds sesame 30%. All the animals consumed diets for a period of 12 weeks.

Animal sacrifice. The rats were anaesthetized at the time of sacrifice using sealed ether-soaked cotton wool inhalation jar between 3 and 5 minutes. The weights of the animals

were taken weekly and before sacrifice (Shittu *et al.*, 2007; Shittu *et al.*, 2008).

Semen collection and semen analyses. The right epididymis of rats in each of two groups were removed and minced into several pieces on a specimen bottle containing normal saline for some few minutes to allow the sperms to become motile and swim out from the right epididymis. The semen was then taken with 1 ml pipette and dropped on a clean slide and covered with cover slips. The slides were placed under light microscope and examined for number and motility sperm according to (Saalu *et al.*, 2006; Saalu *et al.*, 2007a).

Organ harvest and tissue processing for light microscopy. The left Epididymes were carefully dissected out, trimmed of all fat and blotted dry to remove any blood. Then, epididymis were divided into three sections (head, body and tail). The fixed tissues were transferred into boan's solution and then processed for 17.5 h in an automated Shandon processor after which were passed through a mixture of equal concentration of xylene. Following clearance in xylene the sections were then infiltrated and embedded in molten paraffin wax. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicular to the long axes of the epididymes. These sections were designated "vertical sections". randomic sections of 5µm thickness were cut (per 5 sections), floated onto clean slides coated with 2% formaldehyde for proper cementing of the sections to the slides and were then stained with Haematoxylin and eosin stains (Shittu *et al.*, 2006; Shittu *et al.*, 2007).

Determination of morphometric parameters. The diameter (D) of epididymal tubules with profiles that were round or nearly round for each animal was estimated. A mean diameter "D", was taken as the average of two diameters, D1 and D2 (where D1 is the short axis while D2 is the long axis; both D1 and D2 are perpendicular to each other). D1 and D2 were considered only when the ratio of D1+D2/2 (Shittu *et al.*, 2009a; Shittu *et al.*, 2006).

Serum hormonal assay. Serum FSH and LH concentration were estimated with the kit monobind from central laboratory of kashan by ELISA technique (Shittu *et al.*, 2006), while, serum Testosterone estimation was done with the kit monobind using Chemo-Luminance Method.

Statistical analysis. The data were expressed in Mean ± S.E.M (Standard Error of Mean) statistical analyses were done by using the student t-test and ANOVA as the case may be with input into SPSS 12 software microsoft computer (SPSS, Chicago, Illinois). We considered differences significant at P<0.05.

RESULTS

Body weight. The body weight gain during the treatment period did not differ significantly among groups. Moreover, no clinical and behavioral changes were observed in the animals treated with the sesame seed (Table I). Right epididyme sperm motility and count

The mean epididymal sperm motility and count of the experimental group was significantly higher than control group (Table I).

Table I. Effects of the sesame seed on body weights (g), sperm motility and count in right epididymal of wistar male rats treated during 12 weeks.

Parameters	Control	Experimental
Body weights (g)	195.88±7.67	194.42±8.04
Count sperm in right epididyme (·10 ⁶)	62.14±3.91	74.23±2.52*
Motility sperm in right epididyme (%)	57.86±3.77	72.31±2.98**

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

Serum hormone (LH, FSH and T) levels. 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 II).

Table II. Effects of the sesame seed on hormones concentration of wistar male rats treated during 12 weeks.

Parameters	Control	Experimental
LH (IU/L)	2.7±0.29	3.5±0.22*
FSH (mIU/L)	3.9±0.44	3.3±0.28
T (nmol/L)	10.88±2.9	8.45±2.9

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

Table III. Effects of the sesame seed on of morphometric parameters in epididyme wistar male rats treated during 12 weeks.

Parameters	Control	Experimental
Diameter tubule in head part (µm)	290.23±11.04	316.43±8.5
Diameter tubule in body part (µm)	284.75±10.32	302.39±8.11
Diameter tubule in tail part (µm)	284.44±8.45	304.87±5.74
Diameter lumen in head part (µm)	231.4±7.06	245.31±8.6
Diameter lumen in body part (µm)	223.64±8.76	240.68±8.43
Diameter lumen in tail part (µm)	226.95±9.14	239.3±7.0
Diameter epithelium in head part (µm)	58.81±5.0	64.01±7.72
Diameter epithelium in body part	61.11±3.65	61.71±2.35
Diameter epithelium in tail part (µm)	60.48±2.4	65.57±4.88

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

Determination of morphometric parameters. The results obtained in Table III showed that the mean epididymal diameter of the tubular, lumen and epithelium in three parts were not significant in two groups.

DISCUSSION

The purpose of the study was to examine the effect of diet contain sesame seed on epididymis histological adult wistar rat. Moreover, there is increasing role of sesame lignans research because of its contribution to medicine and of immense economic value to man. However, sesame being rich in trace elements or minerals, vitamins and antioxidant lignans (phytoestrogens) posses the ability of improving fertility potential of the male reproductive tract (Shittu *et al.*, 2007).

According to our study, epididymal sperm motility and sperm count in experimental group was significantly higher rather than control group.

Scientist reported antioxidants are known to enhance fertility. Most plants rich in antioxidants have been found to increase sperm counts, motility and enhance sperm morphology (Oluyemi *et al.*, 2007). Vitamin C and vitamin E (as a sesame seed antioxidant) are free radical scavengers and they protect sperm against lipid peroxidation. This ability has been reported to increase peripheral testosterone level (Fridovich, 1986). Paulo and colleagues suggested that the daily sperm production as well as the number of sperms in the cauda epididymis were unaffected, suggesting absence of adverse effects on the spermatogenic process. Also treatment with the highest dose of yarrow aqueous extract altered the sperm

morphology of wistar rats (Dalsenter *et al.*, 2004).

Scientist studied on profertility effects of alcoholic extract of Sesame in male sprague dawley rats, they found that there is a significant increase in motility of sperm in the treatment group that received vitamin C. In the absence of vitamin C, decreased motilities were recorded in treatment groups even upon withdrawal from extract when compared with the group (received vitamin C alongside extract) (Ukwenya *et al.*, 2008). Also, ethanolic extract of sesame enhances morphology of the spermatozoa in the epididymis at the time of sacrifice of the animals (Saalu *et al.*, 2007b).

Ofusori *et al.* (2007) evaluated the effect of ethanolic extract of *croton zambesicus* (as a potent antioxidant and free radical scavenger) on the testes of swiss albino mice and reported that there was a significant increase in the sperm concentration, motility, and progressivity in the treated group rather than the control group. This significant increase was observed to be dose dependent *C. zambesicus*. *C. zambesicus* ameliorated the increased free radicals generated by the natural and experimental stress (Ngadjui *et al.*, 2002; Okokon *et al.*, 2005). Although the mechanism of action of the extract for the increased sperm concentration is yet to be elucidated (Ofusori *et al.*). Epithelial cells called spermatogonia to continuously proliferate to replenish themselves and to differentiate through definite changes of development (Guyton & Hall, 2000). This could be due to the influence of the extract on the mitochondria in the body of the tail of the spermatozoon to synthesize energy in the form of adenosine triphosphate (Guyton & Hall).

According to our study, LH levels significantly increased in experimental group compared to control but significant changes in FSH and testosterone levels were not observed in both groups.

Dahia & Roa (2006) reported that the full fertilizing ability of released spermatozoa is dependent on series of morphological, functional and biochemical changes during the transit through the epididymis, regulated by Androgens testosterone, DHT and estrogens. Additionally, two hormones FSH and LH, are also involved in the processes. In male LH and its receptors (LHR) are necessary for regulate the morphology epithelial cells of the epididymis and epididymal steroidogenesis. And also they showed for the first time that the FSH receptor is also expressed in epithelial cells of the cauda epididymis of rat and monkey.

The examinations of FSH receptor knockout mice (FORKO) demonstrate defects in sperm development, leading to the poor quality sperm production (Krishnamurthy *et al.*, 2000; Sairam & Krishnamurthy, 2001; Grover *et al.*, 2005). The reduction of the various morphometric parameters of caput and corpus epididymides was thought to be dependent on the reduction of testosterone in the serum of FORKO mice and abnormal Sertoli cell functions (Krishnamurthy *et al.*, 2001a; Krishnamurthy *et al.*, 2001b).

Epididymis is also richer in androgen receptor, the site for action of the testosterone, dihydrotestosterone and probably estradiol (Oliveira *et al.*, 2003). Shittu *et al.*, (2007) suggested that Testosterone level in the high dose sesame was significantly higher than the control and the low testosterone level observed in the low dose group could

be due to the fact that some of the testosterone were aromatized to estradiol and/or converted to dihydrotestosterone by the aromatase and reductase enzymes present within the epididymis. Moreover, Huang *et al.*, (1987) demonstrated that as little as 25% of normal testicular testosterone concentration is sufficient to support all stages of spermatogenesis. Shittu *et al.*, (2007) reported that the low dose sesame will make available less endogenous estradiol and compete less, although there is synergism at this level between the testosterone and estradiol to favour spermatogenesis. However the high dose will cause more estradiol production and compete more with dihydrotestosterone for aromatization to occur in its favour if possible. The low testosterone observed here is not as a result of destruction of the Leydig cells but a reflection of the complex hormonal interplay at the level of the hypothalamic-pituitary-testicular axis (Shittu *et al.*, 2007).

Shittu *et al.*, (2009b) who evaluated that mesterolone (proviron) induces low sperm quality with reduction in sex hormone profile in adult male Sprague Dawley rats testis, concluded that testosterone and FSH level in the proviron group was significantly lower than control and it does match to our results, the result obtained from these studies with our results are in the type of diet, 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. 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. The histology features of cauda epididymis were not affected in treated mice, except that there occurred a non significant decrease in the tubular diameter and tubular epithelial height as compared to controls.

The results of the study showed no significant body weight gain, also like in other studies where no significant differences in the animal body weights were observed (Awoniyi *et al.*, 1997). Ukwenya *et al.*, that profertility effects of alcoholic extract of sesame in male sprague-dawley rats studied, they found that the effects of ethanolic extract of beniseed (sesame) at 3000 mg/kg body weight, with vitamin C administered as adjuvant, have shown that beniseed has a potential to increase mean body weights of rats. This is most due to the high fat contents of the seed. Fats are stored in the form of Triacylglycerol in adipose tissues in mammals via lipogenetic pathways. The increased weight gain is in line with the work of Shittu *et al.*, (2007) who also recorded a dose-dependent increase in weight gain upon administration of 14.0 mg/kg and 28 mg/kg body weight of aqueous extract of sesame to rats for six weeks.

Shittu *et al.*, (2007) have suggested that evidence of significant weight gain was observed in all the animals. The weight gain observed in the treated groups were dose dependent such that weight gain in the high dose was more than that in the low dose. However, the low dose weight was significantly lower. However, the results are in disagreement with those of Shittu *et al.*, (2007) study, in that, they found that evidence of significant weight gain was observed in all the animals.

CONCLUSION

This is the first study which has evaluated the histology effect of sesame seed on epididym of adult wistar rat. It can be concluded that sesame seed could improve sperm motility and sperm count, and also it may increase LH. But diet contains sesame seed did not affect on epididymal tissue and body weight.

MAHABADI, J. A.; KHODAYARI, M.; BAFRANI, H. H.; NIKZAD, H. & TABERIAN, A. Efecto de la dieta con semillas de sésamo sobre la histología del epidídimo de la rata adulta. *Int. J. Morphol.*, 31(1):254-263, 2013.

RESUMEN: La sesamina es un importante componente de los lignanos en las semillas de sésamo, y se considera un factor clave en una serie de efectos beneficiosos para la salud humana. La ingesta de sésamo podría mejorar y aumentar la reserva de espermatozoides en el epidídimo en ratas macho adultas Sprague Dawley. El presente estudio tuvo como objetivo investigar los efectos de una dieta que contiene semillas de sésamo sobre la histología del epidídimo de rata Wistar adultas. Treinta ratas macho fueron divididas en dos grupos de 15 animales. El grupo con régimen especial recibió una dieta que contuvo 30% de semillas de sésamo, mientras que el grupo control recibió una dieta estándar durante 12 semanas. El epidídimo del lado derecho fue removido y se cortó y maceró en varios trozos sobre un recipiente de vidrio conteniendo una solución salina normal, durante algunos minutos para permitir que los espermatozoides se volvieran móviles y nadaran fuera; luego los parámetros espermáticos fueron analizados. El epidídimo del lado izquierdo se dividió en tres secciones y se fijó en solución de Bouin para análisis adicionales. Las concentraciones séricas de FSH y LH se estimaron mediante la técnica ELISA, y la concentración de testosterona mediante el método quimioluminencia. El peso corporal obtenido durante el período de tratamiento no difirió significativamente entre los grupos. La motilidad media de espermatozoides del epidídimo y recuento del grupo experimental fue significativamente mayor que el grupo control. Los niveles de LH fueron significativamente mayores en el grupo experimental; no se observaron cambios significativos en los niveles de FSH y testosterona en ningún grupo. Los resultados obtenidos mostraron que el diámetro medio del epidídimo, lumen y epitelio en tres partes diferentes no mostraron cambios significativas en ambos grupos. Se puede concluir que las semillas de sésamo mejoran los parámetros espermáticos (motilidad y recuento), y también puede aumentar la LH. Sin embargo, la dieta que contiene semillas de sésamo no afectó el tejido del epidídimo y peso corporal.

PALABRAS CLAVE: Dieta; Epidídimo; Semilla de sésamo; Semen; Hormonas.

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