

Full Length Research Paper

# Effect of sesame seed rich diet on the structure of the epididymis

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Sesamin is a major lignan constituent of sesame seed and considered as a key factor in a number of beneficial effects on human health. The intake of sesame leaves has been shown to improve and increase epididymal spermatozoa reserve in adult male Sprague Dawley rats. The aim of the study was to determine the effect of a diet containing sesame seeds on epididymis histological structure of the adult wistar rat. Thirty adult male rats were divided into two groups consisting of 15 rats each. The regime group received a diet containing 30% sesame seeds, while the control group received a standard diet for 12 weeks. The right epididymis was removed and minced into several pieces in a specimen bottle containing normal saline for a few minutes to allow the sperm to swim out. Sperm parameters, sperm count and motility was determined. The left epididymis was divided into three sections and fixed into bouin's solution for histological evaluations. Serum Follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were estimated by Enzyme-linked immunosorbent assay (ELISA) techniques while testosterone concentration was determined using a Chemo-luminescence method. The body weight gain during the treatment period did not differ significantly between the two groups. The mean epididymal sperm motility and count of the experimental group was significantly higher ( $p < 0.05$ ) when compared to the control group. LH levels were significantly increased in the experimental group compared to control group. No significant changes in FSH and testosterone levels were reported. The mean epididymal diameter of the tubular, lumen and epithelium in three parts were not significantly different ( $p > 0.05$ ) when the regime group was compared to the control group. From our study, we can conclude that the consumption of sesame seeds improves sperm parameters (motility and count) and increases LH levels. Sesame seeds had no effect on epididymal tissue and body weight of the rats.

**Key words:** Diet, epididymis, sesame seed, sperm, hormone

## INTRODUCTION

The function of the epididymis, including production of the epididymal specific micro-environment is necessary for the maturation, storage, and survival of spermatozoa which 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 and 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 which is cultivated widely in tropical, subtropical, and southern temperate regions (Chakraborty et al., 2008).

Sesamin is a major lignan constituent of sesame seeds and considered as a key factor in a number of beneficial effects on human health. These benefits include anti-cancer properties (Yokota et al., 2007), anti-hypertensive properties (Miyawaki et al., 2009), anti-inflammatory properties (Jeng et al., 2005), anti-oxidative properties (Nakano et al., 2003), cholesterol-lowering activity (Chen et al., 2005), enhancement of hepatic fatty acid oxidation and alcohol metabolism (Tsuruoka et al.,

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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). This plant source is now increasingly being incorporated into the human diet worldwide because of their reported health benefits (Shittu et al., 2009). Shittu et al. (2007) reported that sesame leaves intake improve and increase epididymal spermatocytes reserve in adult male Sprague Dawley rats. Sesame lignan, such as: sesamin, sesamol, sesaminol, sesamolol, pinorsinol, sesamol and gamma-tocopherol are isolated from *S. indicum* and *S. radiatum* seeds and have more anti-tumorigenic, estrogenic or anti-estrogenic and antioxidant features compared with other plant species (Jeng and Hou, 2005).

In terms of phytochemicals, 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 (Konan et al., 2008). Minerals or trace elements such as calcium, iron, magnesium, zinc, copper and phosphorus exist in this plant (Shittu et al., 2006). Approximate analysis of sesame seed has made it clear that the seeds contain 50 to 60% oil, 8% protein, 5.8% water, 3.2% crude fiber, 18% carbohydrate and 5.7% ash (Obiajunwa et al., 2005). It has been reported by Shittu and Shittu (2012) that phytochemicals in sesame leaves had an effect on improving fertility potential and antimicrobial activities, as well as significantly increasing the mean epididymal diameter and volume density of the tubular lumen in low dose sesame group. When compared to the control group. In addition, the consumption of sesame leaves extract enhanced the quality of the spermatozoa produced with improvement in the storage capacity of the epididymis for these spermatozoa in a dose related manner (Shittu and Shittu, 2012).

The influence of lead and zinc on the rat male reproductive system at the biochemical and histopathological levels has been evaluated (Obiajunwa et al., 2005). These authors reported that zinc (an element of sesame seed) supplementation to lead-exposed rats had a protective effect. Zinc could compete significantly and effectively reduce the availability of lead binding sites (Batra et al., 2001). The aim of our study was to determine the effect of a sesame seed rich diet on the structure of the epididymis using histomorphometric methods as well as on hormone (LH, FSH and testosterone) levels.

## MATERIALS AND METHODS

### Animal experiment

Thirty mature and healthy adult male wistar rats weighing between 190 to 210 g were procured from Kashan University, College of

Medicine and Kashan-Iran from the period of 2010 to 2011. They were housed in well ventilated wire-wooden 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 to 50%). The animals were allowed to acclimatize for a period of 7 days before treatment during the experiments. During the next 12 week period, they were fed with standard rat chows supplied, and water was available *ad libitum* (Shittu et al., 2007, 2008). All of the clinical trials conducted on animals were approved by Animal Care Committee of Kashan University of Medical Sciences.

### Experimental design

An experimental study was designed in that, two types of rats (A and B) were randomly selected and were assigned into either the experimental (n = 15) or the control groups (n = 15). Group A served as the control while B constituted the treated group. Group A animals received normal diet while group B received normal diet (70%) plus 30% sesame seeds. All the animals consumed diets for a period of 12 weeks.

### Animal sacrifice

The rats were anaesthetized at the time of sacrifice using a sealed inhalation jar with ether-soaked cotton wool for 3 and 5 min. The weights of the animals were taken weekly and before sacrifice (Shittu et al., 2008).

### Semen collection and semen analyses

The right epididymis from rats of each of the two groups were removed and minced into several pieces in a specimen bottle containing normal saline. It was allowed to stand for a few minutes to allow the sperm to swim out into the saline medium. The semen was collected with a 1 ml pipette and dropped on a clean slide and covered with slips. The slides were placed under a light microscope and examined for the sperm number and motility (Saalu et al., 2006, 2007a).

### Organ harvest and tissue processing for light microscopy

The left epididymis was carefully dissected, trimmed of all fat and blotted dry to remove any blood. The epididymis was divided into three sections (head, body and tail). The fixed tissues were transferred into bouin's solution and then processed for 17.5 h in an automated Shandon processor after which the tissues 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 were cut using a rotary microtome which was orientated perpendicularly to the long axes of the epididymis. These sections were designated "vertical sections". Ranomic sections of 5 µm 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, 2007).

### Determination of morphometric parameters

The diameter (D) of the 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

**Table 1.** Effects of sesame seeds consumption on body weights, sperm motility and count in epididymis of wistar male rats treated during 12 weeks.

Parameter	Control	Experiment
Body weights (g)	195.88±7.67	194.42±8.04
Sperm count (×10 <sup>6</sup> )	62.14±3.91	74.23±2.52*
Sperm motility (%)	57.86±3.77	72.31±2.98**

Results expressed in mean ± SEM. \*Indicates P < 0.01; \*\*indicates P < 0.006.

**Table 2.** Effects of the sesame seeds consumption on serum hormone concentrations of wistar male rats.

Parameter	Control	Experiment
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 ± SEM. \*indicates P < 0.05. LH = luteinizing hormone, FSH = follicle stimulating hormone, T = testosterone.

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 is D1 + D2/2 (Shittu et al., 2006).

#### Serum hormonal assay

Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were estimated using an ELISA method with the monobind kit from Central Laboratory of Kashan (Shittu et al., 2006). Serum testosterone (T) levels were determined using a monobind kit with a Chemo-luminance method.

#### Statistical analysis

The data were expressed as Mean ± SEM (Standard Error of Mean). Statistical analyses were done using the student t-test and Analysis of variance (ANOVA) with input into Statistical Package for the Social Sciences (SPSS) 12 software Microsoft computer (SPSS, Chicago, Illinois). We considered significant differences at P < 0.05.

## RESULTS

### Body weight

The body weight gain during the treatment period did not differ significantly between the two groups. Moreover, no clinical and behavioral changes were observed in the animals treated with the sesame seeds (Table 1).

### Epididymal sperm motility and count

The mean epididymal sperm motility and count of the

experimental group was significantly higher than control group (Table 1).

### Serum hormone levels

LH levels were significantly increased in the experimental group compared to the control group; however no significant changes in FSH and testosterone levels were observed (Table 2).

### Determination of morphometric parameters

The results in Table 3 indicate that the mean epididymal diameter of the tubular, lumen and epithelium in three parts were not significantly different (P > 0.05) between the two groups. Measuring the mean diameter of epididymal tubular was done by Zeiss optical microscope with a magnification of ×10.

## DISCUSSION

The purpose of the study was to examine the effects of a diet containing sesame seeds on the histological profile of the epididymis of the adult wistar rat. Moreover, there is an increasing role of sesame lignans research because of its contribution to medicine and of its immense economic value to man. However, sesame being rich in trace elements or minerals, vitamins and antioxidant lignans (phytoestrogens) possesses the ability to improve fertility potential in the male (Shittu et al., 2007).

According to our study, epididymal sperm motility and sperm count in experimental group was significantly higher in comparison to control group. It has been reported that antioxidants have the ability to enhance fertility (Ganong, 2003). Most plants rich in antioxidants have been found to increase sperm counts, sperm motility and enhance sperm morphology (Oluyemi et al., 2007). Vitamins C and E (as a sesame seed antioxidant) are free radical scavengers and they protect sperm against lipid peroxidation with an increase in peripheral testosterone levels (Fridovich, 1986). Dalsenter et al. (2004) suggested that the daily sperm production, as well as the number of sperms in the caudal epididymis were unaffected by aqueous crude extract of *Achillea millefolium* leaves, suggesting absence of adverse effects on the spermatogenic process. Treatment of wistar rats with the highest dose of yarrow aqueous extract altered the sperm morphology (Dalsenter et al., 2004).

Pro-fertility effects of the alcoholic extract of Sesame in male Sprague Dawley rats were investigated. These authors found a significant increase in motility of sperm in the treatment group that received vitamin C (Saalu et al., 2007b). In the absence of vitamin C, decreased sperm motilities were recorded in treatment groups even upon withdrawal from the extract, when compared to the group

**Table 3.** Effects of the sesame seeds consumption on morphometric parameters in epididymis of male wistar rats.

Parameters ( $\mu\text{m}$ )	Control	Experiment
Tubule diameter in head part	290.23 $\pm$ 11.04	316.43 $\pm$ 8.5
Tubule diameter in body part	284.75 $\pm$ 10.32	302.39 $\pm$ 8.11
Tubule diameter in tail part	284.44 $\pm$ 8.45	304.87 $\pm$ 5.74
Lumen diameter in head part	231.4 $\pm$ 7.06	245.31 $\pm$ 8.6
Lumen diameter in body part	223.64 $\pm$ 8.76	240.68 $\pm$ 8.43
Lumen diameter in tail part	226.95 $\pm$ 9.14	239.3 $\pm$ 7.0
Epithelium diameter in head part	58.81 $\pm$ 5.0	64.01 $\pm$ 7.72
Epithelium diameter in body part	61.11 $\pm$ 3.65	61.71 $\pm$ 2.35
Epithelium diameter in tail part	60.48 $\pm$ 2.4	65.57 $\pm$ 4.88

Results expressed in mean  $\pm$  SEM.

that received vitamin C alongside extract (Ukwenya et al., 2008). Ethanolic extract of sesame was shown to enhance 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 effects of ethanolic extract of croton zambesicus (as a potent antioxidant and free radical scavenger) on the testes of Swiss albino mice. A significant increase in the sperm concentration, motility, and progressivity in the treated group was observed. *C. zambesicus* ameliorated the increased free radicals generated by the natural and experimental stress (Ngadjui et al., 2002; Okokon et al., 2005). The mechanism of action of the extract for the increased sperm concentration is yet to be elucidated (Ofusori et al., 2007). Spermatozoa continuously proliferate to replenish themselves and differentiate through definite changes of development (Guyton and Hall, 2000). The extract could possibly have an effect on the mitochondria in the body of the tail of the spermatozoon influencing its ability to synthesize energy in the form of adenosine triphosphate (Guyton and Hall, 2000).

The data obtained in our study show that LH levels were significantly increased in the experimental group when compared to control but significant changes in FSH and testosterone levels were not observed in both groups. Dahia and Roa (2006) reported that LH and its receptors (LHR) are necessary for regulation of the morphology of epithelial cells of the epididymis and epididymal steroidogenesis. It has been shown for the first time that the FSH receptor is also expressed in epithelial cells of the caudal epididymis of rat and monkey (Dahia and Rao, 2006).

The epididymis is also richer in androgen receptors, the site for action of the testosterone, dihydrotestosterone and probably estradiol (Oliveira et al., 2003). Shittu et al. (2007) suggested that testosterone levels in the high dose sesame group was significantly higher than the control and the low testosterone level observed, which 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 was sufficient to support all stages of spermatogenesis. Shittu et al. (2007) reported that the low dose sesame will reduce endogenous estradiol with less competition, although there is synergism at this level between the testosterone and estradiol to favour spermatogenesis. However the high dose of sesame could possibly result in more estradiol production with more competition with dihydrotestosterone for aromatization to occur in its favour. The low testosterone observed in this study is not 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. (2009) observed the effects of mesterolone (proviron) to induce low sperm quality with a reduction in sex hormone profile of the testis of adult male Sprague Dawley rats. It was concluded that testosterone and FSH levels in the proviron group was significantly lower than the control group. These results are in contradiction to our findings and could be attributed to variations in the type of diet, type of rats and duration of using a particular diet. However, it was found that FSH had a synergistic effect with testosterone hormone and stimulating synthesis of the androgen receptor at the receptor level. According to a study by Shittu et al. (2008), FSH concentration decreased in the group that received the high dose liquid extract of sesame leaves group compared to the control group. The histological features of the caudal epididymis were not affected in treated mice, except there was a non significant decrease in the tubular diameter and tubular epithelial height as compared to controls (Shittu et al., 2008).

The results of the study indicate that no significant changes in body weight gain were evident. These were confirmed in other studies where no significant differences in the animal body weights were observed

(Awoniyi et al., 1997). Ukwenya et al. (2008) demonstrated the pro-fertility effects of the alcoholic extract of sesame in male Sprague Dawley rats studied. The effects of the ethanolic extract of beniseed (sesame) at 3000 mg/kg body weight, with vitamin C administered as adjuvant, had the potential to increase the mean body weights of rats. This is mostly due to the high fat content of the seeds. 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 and 28 mg/kg body weight of aqueous extract of sesame to rats for six weeks.

Shittu et al. (2007) have suggested that significant weight gain was observed in all the animals. The weight gain observed in the treated groups was 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) where they reported significant weight gain in all the animals.

## Conclusion

This is the first study which has evaluated the histology effect of sesame seed on epididymis of adult Wistar rat. It can be concluded that sesame seed could improve sperm motility and sperm count, and also it may increase LH. A diet rich in sesame seed did not have any effect on epididymal tissue and body weight of animals.

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