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Full Length Research Paper

Sesame leaves intake improve and increase epididymal spermatocytes reserve in adult male Sprague Dawley rat

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Increasing concern has been expressed about the potential effects of both synthetic and natural estrogenic endocrine disruptors (EEDs) on human reproductive health in our environment in the last decade. However, little attention is paid to histomorphometric structural changes of the epididymis. We aim to evaluate the chronic exposure effects of phytoestrogens found in aqueous extract of *Sesame radiatum* leaves on the male Sprague Dawley (SD) rats' epididymes. Thirty adult male SD rats were randomly divided into three groups (2 treated and 1 control groups respectively). In the treated groups, a single daily dose of aqueous leaves extract of *S. radiatum* (14.0 mg/kg and 28.0 mg/kg body weight) were administered via gastric garvage, while, equal volume of normal saline was administered in control group for six weeks. Histomorphometric study of the epididymal tissues and hormonal assay were analyzed using SPSS software and $P < 0.05$ was considered statistically significant. Significant ($P < 0.05$) body weight gain in a dose dependent was observed in all the animals. Also, there was significant weight gain in both raw weight and relative organo-somatic weight of the epididymis per 100 g body weight. However, the weight gain was more in the high dose than the low dose group. The epididymal lumen appeared wider and fuller with spermatocytes when compared to the control. There is significant ($P > 0.05$) increases in testosterone level compared to control, however, the low dose was also significantly lower than the control. Sesame improves the storage capacity for the spermatozoa in the epididymis in a dose related manner.

Key words: Epididymis, sesame leaves, histomorphometric study.

INTRODUCTION

Increasing concern has been expressed about the potential effects of both synthetic and natural estrogenic endocrine disruptors (EEDs) on human reproductive health as evidence by its adverse effect on both the male reproductive tract and sperm quality in wildlife species and humans in our environment in the last decade (Vos et al., 2000). 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 Shak-

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kebeak,1993; Sharpe, 2003; Izegbu et al., 2005; Shittu, 2006).

Sesame plant is one of the richest food source of lignans, a major type of phytoestrogens known to man since the dawn of civilization (Thompson et al., 1991) and is increasingly being incorporated into human diets because of its reported health benefits. However, sesame lignans such as sesamin, episesamin, sesamolin, and ɣ-tocopherol isolated from *Sesamum indicum* and *Sesamum radiatum* seeds among other species have been implicated as having anti-tumorigenic (Hirose et al., 1992), estrogenic and/or anti-estrogenic (Collins et al., 1997; Shittu, 2006) and antioxidant (Kang et al., 1999; Hou et al., 2004; Shittu, 2006) properties. Moreover, the ROS scavenging moiety of sesame lignans may contribute important components which prevent body cells from the free radical injury (Jeng et al., 2005).

Sesame is reputed in folk medicine in Africa and Asia. All parts of the plant are useful.

Sesame plant especially the seed, oil and leaves are consumed locally as a staple food by subsistence farmers in South-West and Middle Belt areas of Nigeria (Akpan –Iwo et al., 2006) and this may account for the high fecundity among the adult male population in these areas (Shittu, 2006). In the South-Western Nigeria, decoction of the leaves is used for the treatment of bruised or erupted skins, catarrh and eye pains. However, leaves decoction has been found to have antimicrobial effects (Shittu et al., 2006; Bankole et al., 2007).

The spermatocytes released from the testis, upon reaching the epididymis will undergo additional physiological maturation (capacitation) and gain fertilizing capacity and motility. However, sperm maturation in the epididymis involves various morphological and biochemical changes with the initiation of progressive motility and acquisition of fertilizing ability. The acquisition of sperm motility is a key element of epididymal sperm maturation (Orgebin-Crist, 1967), which occurs in the microenvironment provided by the epididymal secretory products such as Sialic acid, acetyl carnitine, Glyceryl phosphoryl choline (GPC) among others, to help maintain the osmolarity of epididymal luminal fluid (Wales et al., 1966), thereby serving as stabilizer of the spermatozoal membrane (Scott et al., 1963), and playing a vital role in the metabolism of spermatozoa after capacitation (Mitra and Chowdhury, 1994).

However, estrogen (E2) has been implicated to regulate ions transport and luminal fluid reabsorption in the efferent ductules of the male. Since, most of the testicular fluid (about 96%) is reabsorbed by the non-ciliated cells in the efferent ductules (Clulow et al., 1998) and without this re-absorption, the sperm will remain diluted and incapable of maturation in the epididymis and as such any blockage in the estrogen receptor's function may result in infertility. Moreover, estrogen is responsible also for maintaining the differentiated epithelial morphology through an unknown mechanism. Thus, estrogen or its receptor is important for normal functioning of the male reproductive tract in numerous species (Hess and Carnes, 2004).

As a result of paucity of knowledge, we aim to carry out this study on the chronic toxicity effect of aqueous extract of *S. radiatum* leaves on the adult male Sprague Dawley (SD) rat epididymis using histomorphometric study and hormonal assay.

MATERIALS AND METHODS

Collection of plant materials

Sesame plants (*S. radiatum*, Schum and Thonn - Pedaliacaea family) were bought from a vendor in Agege market, Lagos. The plant was authenticated by the herbarium section of Forestry Institute of Research (FRIN) with FHI # 107513 on the 5th of August, 2005 (Shittu et al., 2006). Voucher specimens were deposited in Botany Departments of University of Ibadan and Lagos State University, respectively.

Preparation of extracts

The leaves of the plants were air dried for 2 weeks and powdered. For the preparation of the aqueous extraction of sesame leaves, 100 g of the powdered leaves was added to 1.0 litre of distilled water at a ratio of 1:10 in a beaker and allowed to boil to boiling temperature after intermittent stirring on a hotplate for one hour. The decoction was filtered into another clean beaker using a white sieve clothing material and the filtrate evaporated at 50^{o}C to dryness in a desiccator to produce a black shinning crystal residue form with a yield of 83% w/w of the extract. The crude extract was kept in the refrigerator (4^{o}C) before being reconstituted and used for the *in vivo* study.

To prepare the ether extracts, 100 g of the powdered leaves were extracted with 500 ml of 40% diethyl ether for 72 h with Soxhlet equipment using modified method of Alade and Irobi (1993).

Phytochemical screening using gas chromatography-mass spectral

Gas chromatography of crude ether extract of sesame leaves was performed using a Hewlett Packard GCD system (model 6890), equipped with a flame ionization detector and injector MS transfer line temperature maintained at 230°C respectively as described in our previous study (Shittu et al., 2006). Compound identification was accomplished by comparing the GC relative retention times and mass spectra to those of authentic substances analyzed under the same conditions, by their retention indices (RI) and by comparison to reference compounds (Shittu et al., 2006).

Animal experiment

Thirty mature and healthy adult male Sprague Dawley rats weighing 120 to 216 g were procured from Ladoke Akintola University, College of Medicine, Ogbomosho and 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^{o}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 they were fed with standard rat chows/pellets supplied by Pfizer Nigeria Limited and water *ad libitum*. Individual identification of the animal was made by ear tags.

Table 1. Average weekly body weight of animal.

Weight	A (Control)	B (High dose)	C (Low dose)
Initial (pre-experiment) (g)	127.3 ± 5.55	206.2 ± 6.45	186.3 ± 1.99
Final (post- experiment) (g)	185.2 ± 11.05*	248.2 ± 14.40*	219.8± 4.47*
weight gain (g)	58.5 ± 5.50*	42.0 ± 7.95*	33.4± 2. 48*

Values are mean ± S.D. *P < 0.05 was considered significant.

Table 2. Summary of weight (g) of epididymis.

Group	Raw weight (g)	Epididymo-somatic weight (wt/100 g bwt)
Group A (control)	0.55 ± 0.03	-
Group B (high dose)	0.75 ± 0.01*	0.30 ± 0.00*
Group C (low dose)	0.57 ± 0.01*	0.26 ± 0.01*

Values are mean ± S.D.
*P < 0.05 was considered significant.

The rats were randomly divided into three groups (A to C) comprising of ten rats each. The group A served as the control while B and C constituted the treated groups. The animals in group A received equal volume of 0.9% (w/v) normal saline daily while group B received aqueous extract of sesame leaves at 14.0 mg/kg body weigh /day(half the group B dose). The animals in group C were given aqueous extract of sesame at 28.0 mg/kg body weight/day (twice the group B dose). All the doses were given via gastric gavage (oro-gastric intubation) daily for a period of 6 weeks. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the decalration of Helsinki and the guiding principles in the care and use of animals (American Physiological Society, 2002) and were approved by the departmental committee on the use and care of animals. All animals were observed for clinical signs of drug toxicity (such as tremors, weakness, lethargy, refusal of feeds, weight loss, hair-loss, coma and death) throughout the duration of the experiment.

The rats were anaesthetized at the time of sacrifice by being placed in a sealed cotton wool soaked chloroform inhalation jar between 0900 and 1100 h done the following day after the termination of the experiment following over night fasting of the animals. The weights of the animals were taken weekly and before the sacrifice. The epididymes were carefully dissected out, trimmed of all fat and blotted dry to remove any blood. Their weights were noted and volume measured by water displacement with the aid of a 10 ml measuring cylinder. Later, the sizes (length and width) were recorded by use of a sliding gauge (d = 0.1) before being fixed in freshly prepared 10% formol saline solution. The fixed tissues were transferred into graded alcohol and then processed for 17.5 h in an automated Shandon processor after which was 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 microtone were orientated perpendicular to the long axes of the epididymes. These sections were designated “vertical sections”. Serial sections of 5 µm thickness were cut, floated onto clean slides coated with Mayer’s egg albumin for proper cementing of the sections to the slides and were then stained with haematoxylin and eosin as previously described (Shittu, 2006).

Hormonal assay

The estimation of testosterone was carried out using the procedure enclosed with the kit purchased from Amersham International Plc. (UK) by ELISA technique as previously described (Shittu, 2006). The inter- and intra-assay coefficients of variation for the testosterone were <15%.

Statistical analysis

The weight data were expressed in mean ± S.D. (standard deviation) while other data were expressed as 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). Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

The GC/MS showed the presence of essential oils mainly the carboxylic phenolic groups such as (sesamol, sesamin) and other compounds such as thiazole, pyrroles, disulphide, ketones and aldehyde (Shittu et al., 2006).

No obvious toxicity signs such as weakness, lethargy, tremors, refusal of feeds, weight loss, hair loss, coma and death were seen in any of the animals. However, most of the animals exhibited calmness; improve appetite for food and water and general sense of well-being, all through the duration of the study. Evidence of significant ($P < 0.05$) 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 as observed in Table 1. The epididymal weight increased significantly ($P < 0.05$)

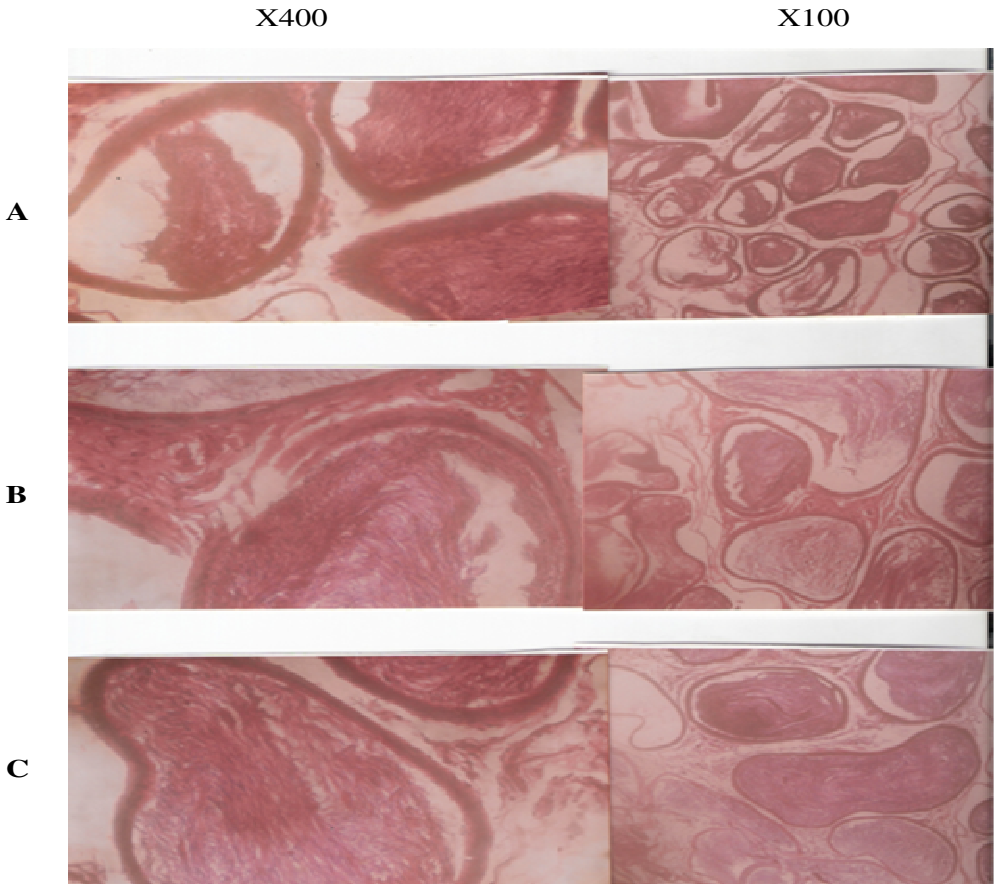

Figure 1. Showing the micrographs of the animals. A. The top micrographs showed the control group at X400 and X100 magnification. B. The middle micrographs showed evidence of spermatocytes fullness in the varying tubular lumen of the low dose epididymes observed at X400 and X100 magnification. C. The lower micrographs showed similar features like the low dose groups but with a fuller tubular lumen seen when compared to the control groups (at the top) at both X400 and X100 magnifications.

Table 3. Summary of hormonal profile of the animals.

Hormone (ng/ml)	Group A (Control)	Group B (High dose)	Group C (Low dose)
TEST	0.8 ± 0.03	0.9 ± 0.2*	0.1 ± 0.0*

in the high dose as compared to the low dose with no significant weight difference in the relative epididymosomatic weight (weight/100 g body weight) of the high dose compared to control. However, the low dose weight was significantly lower as observed in Table 2. The epididymal lumens in the sesame treated were wider, fewer with irregular tubular formation and significantly filled with spermatocytes when compared to the control group which are relatively numerous with smaller luminal sizes as shown in the Figure 1.

There is significant ($P > 0.05$) increases in testosterone level compared to control, however, the low dose was also significantly different from the control as shown in Table 3.

This is the first study, to our knowledge, to look at the role of sesame lignans on the male reproductive tracts after extensive literature review. There is increasing role of sesame lignans research contribution to medicine. However sesame, being rich in trace elements or minerals, vitamins, antioxidant lignans (phytoestrogens), have the ability of improving fertility potential of the male reproductive tract but its mechanism of action need to be elucidated in this study.

The beneficial effects derived from the high intake of fruits and vegetables on the various metabolic disease conditions (such as diabetes mellitus, obesity, heart diseases and cancer), that may impact on one's reproductive life may not be due to the effect of their well charact-

erized antioxidants, such as β-carotene, vitamins C and E only, but rather to some other antioxidants or non-antioxidant phytochemicals or by an additive actions of the different compounds present in foods (fruits and vegetables) such as alpha-linolenic acid (poly unsaturated fatty acids), various phenolic compounds (sesamin, sesamol) and fibres, which are present in sesame leaves for example (Shittu et al., 2006).

All the animals that were used in this study, irrespective of their aggressive nature, exhibited calmness (non-aggressive state) during treatment. More so, there was a general state of well-being observed in all the animals during the whole experimental period. This may reflect the positive effect of some of the active agents present in the plant on their neuropsychological and neuro-endocrine pathways. The sesame-treated rats showed significant weight gain ($P < 0.05$, using ANOVA), unlike in other studies where no significant difference in the animal weights was observed (Awoniyi et al., 1997).

The relative epididymal weight difference is a reflection of the site of action of the sesame estrogenic lignans which may also be hormonally influenced. Since the efferent ductile and epididymis of rats are rich in estrogens recaptors; α and β sub-types (Hess et al., 1997; Hess and Carnes, 2004) just like any other part of the body such as uterus and mammary glands among others (Williams et al., 2000; Nie et al., 2002), any disruption of these receptors as a result of abnormalities in the efferent ductules or epididymis have been hypothesized to lead to impairments of male fertility in mice (Hess, 1997; Hess and Carnes, 2004). However, efferent ductile or epididymis is also richer in androgen receptor, the site for action of the testosterone (TT), dihydrotestosterone (DHT) and probably estradiol (E2) (Oliveira et al., 2003).

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 and that consistency in testosterone concentration is important for normal spermatogenesis to occur as evidence by the low dose epididymal lumen with fuller spermatocytes compared to control in the present study.

Moreover, sesame phytoestrogenic lignans will tend to promote aromatization of testosterone to estradiol, such 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, reverse is the case for the high dose, which 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.

Brown and Chakraborty (1992) have suggested that agent like clomiphene will decrease the synthesis and/or release of gonadotrophins such that decreased serum LH (Luitenising Hormone) and testosterone concentration (ng/ml) were found in male rats. However, low dose estradiol is also needed for spermatogenesis than high dose as evidence in the present study.

Little is known about the mechanism of action of sesame phytoestrogen lignans with antioxidative properties. But, contrary to speculation that phytoestrogen disrupt male reproductive development through mechanisms which are independent of the estrogens receptors, in which there is estradiol (E2)-induced transactivation of the androgen receptor (AR) (Degen and Metzler, 1987). We are able to demonstrate that sesame lignans has no negative impact on the male reproductive tract through its binding on the estrogen receptors but also modulate the activity of the androgen receptor in the epididymis, thereby ultimately influencing the hypothalamic-pituitary-testicular pathway as evidence in this study.

In general, aromatase has not been found in rat testis, efferent ductules, epididymis or vas deferens (Hess and Carnes, 2004). However, various reports are found on the epididymal presence of aromatase in human efferent ductules and proximal epididymis (Carpino et al., 2004) and cultured rat cells, (Wiszniewska, 2002).

We also hypothesized that the action of sesame on the β-estrogens receptors as an agonist is more pronounced than that of the α-estrogens receptors with transactivation of the androgen receptor together with its antioxidative property have all contributed to enhancing spermatogenesis with improve male fertility (through production of quality spermatocytes) found in this study. However, study is on to confirm this hypothesis further.

Conclusion

Sesame improve epididymal sperm reserve with larger spermatocytes being produced in a dose related manner as evidence in this study through a complex hormonal interplay at the level of the male hypothalamic-pituitary-testicular axis and estrogens receptors.

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