Profertility Effects Of Alcoholic Extract Of Sesame In Male Sprague-Dawley Rats

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Citation

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Abstract

The effects of administration of ethanolic extract of sesame and Vitamin C as adjuvant on the reproductive system of male sprague-Dawley rats were exploited in this work. 20 rats were randomized into four groups (Control, T_1, T_2, T_3) of 5 rats each. One of the group serves as control group while the other 3 groups received a sub-lethal dose of sesame extract (3000mg/kg body weight) once in a day for 8 weeks. One of the treatment groups (T_3) also received 2.5mg/kg of Vitamin C for 8 weeks. At the end of the 8 weeks, animals in Control, T_1 and T_3 were sacrificed while T_2 was withdrawn from extract administration for 4 weeks to test for the effect of withdrawal. Sperm counts, Morphology and Motility were quantified and organ and general body weights were measured in a weighing balance. Results of the experiment revealed an increment in the sperm count and sperm percentage motility but a decreased percentage morphology distortion. There were dose-dependent increments in body and organ weight gains, withdrawal notwithstanding. This experiment thus revealed that ethanolic extract of sesame seed possesses potent profertility properties which may be beneficial to those who consume it.

INTRODUCTION

The attention given to various use of phytomedical products are now on the increase. Many researchers now focus on several ways of making infertility a thing of the past as well as effectively controlling unwanted pregnancies. The tradititonal use of Beniseed (English: Sesame; Yoruba: Ekuku; Igbira: Igorigo.) dates back to about 4000 years ago in Babylon and Assyria where it is used to produce Sesame cake, wine, brandy [1]. It is an important oilseed crop believed to have originated from tropical Africa where you have the greatest genetic diversity. It was later taken at a very early date to lndia where a secondary center of diversity developed [2]. The total world crop area under beniseed is about 6million hectares. 66% of this is concentrated in Asia while 25% is planted in Africa (mainly Nigeria, Ethiopia and Sudan) and 8% in America (Venezuela, Mexico, Guatemala and Columbia). The leading world producers are India, China, Mexico and Sudan in Africa. Total annual consumption is about 65% for oil extraction and 35% for food. The sesame oil produced from its seed compares favourably with olive oil as this has been substituted for the latter in many places around the world and especially in Europe. It is also used in sesame cake, salad, wine, brandy, buns and chips [3]. It is expedient to know that some of the potent phytochemical components are antioxidants and steroids. Antioxidants such as sesamin (0.34 to 1.13%), sesamolin (0.13 to 0.58%) and sesamol (liberated from sesamolin by diluting it with mineral acids or by hydrogenation. National Cereals Research Institute have been found to have nutraceutic effects [3,4]. Antioxidants are known to enhance fertility either directly or indirectly. Most plants rich in antioxidants have been found to increase sperm counts, motility and enhance sperm morphology [5,6]. These compounds are capable of removing free radicals from the body system. Vitamins C and E (?-tocopherol) are free radical scavengers and they protect against lipid peroxidation. This ability has been reported to both increase peripheral testosterone level and enhance attenuate testicular toxicity [7]. Vitamins present in sesame include Thiamin (0.98mg/100g), Riboflavin (0.25mg/100g) and Niacin (5.40mg/g). These are all potent antioxidants and are capable of increasing testosterone formation by the interstitial cells of Leydig.

The seed contains fatty and non-fatty acids. The relative amount of each depends upon variety and quality of seed. Fatty acid compositions of typical beniseed oil are 7 to 11% of palmitic acid; 2 to 6% of stearic acid; 32 to 54% of oleic and 39 to 56% of linoleic acid. Other fatty acids occur in amounts less than 1% [3]. Fatty acids such as cholesterol formed from smaller fats are essentially needed in the

formation of testosterone [8] .

Sesame oil-containing diet significantly reduced artherosclerotic lesion formation and plasma cholesterol, triglyceride and low density lipoprotein (LDL) levels in LDL Receptor negative mice [9] .

Sesame seeds have been implicated as having anti-tumorigenic [10] , estrogenic and/or anti-estrogenic [11,12] and antioxidant [13] properties.

The seminal vesicle secretes a yellow viscous fluid containing substances that activate sperm (fructose). This fluid constitutes about 70% of the human ejaculate [14] .

Although the seminal vesicle may not contain factors that are absolutely responsible for fertilization, their secretion still plays an important role in optimizing conditions for sperm viability, motility and survival as well as sperm transport ( ). Testis is the male gonad that produces the spermatozoa through cascade of processes collectively referred to as spermatogenesis. This process is under the influences of testosterone, FSH and LH [5,15] .

The present research aims at evaluating the effects of ethanolic extract of beniseed on sperm parameters and weights of reproductive organs as indices for fertility. The organs tested are testis, epididymis and seminal vesicle.

MATERIAL AND METHODS

ANIMALS

Twenty (20) adult male rats of Sprague Dawley strain (Approx. weight of 200g) were used in this study. They were obtained from Animal house of the faculty of Basic medical sciences, Ladoke Akintola University of Technology, Ogbomoso. They were brought to the animal control room of the Anatomy Department, University of Lagos. The rats were kept in iron cages at controlled room temperature of about 30°C and photo-periodicity of 12L: 12D. They were fed on rat pellet feed obtained from Ladokun feeds, Ibadan and water made available “ad libitum”. They were allowed to acclimatize for two weeks before the commencement of the experiments.

PLANT MATERIAL

Beniseed was purchased locally at Ganmo market, Ilorin, Kwara State. The seed was authenticated at Botany Department, University Lagos, Lagos. Alcoholic extraction and Lethal Dose (LD) estimation of beniseed was done at Pharmacognosy Department, College of Medicine University of Lagos. LD_{50} of beniseed was found to be 5000mg/kg.

EXPERIMENTAL DESIGN

The rats were divided into four (4) groups of 5 rats each. The groups were designated as control (c) and Treatments (T) with subscript 1, 2 and 3. i.e.

C = Control group, T_1 = Treatment group 1, T_2 = Treatment group 2 and T_3 = Treatment group 3.

Each of the rats in the treatment groups (T_1,T_2,T_3) received 3000mg/kg body weight of beniseed extract daily between 07.00 to 8:00 hours for a period of 8 weeks. $T_{3\ also}$ received 2.5mg of Vitamin C (made by Tuyil Pharmaceutical Company Ilorin, Kwara State) in addition to beniseed extract. The animals were weighed once a week. After the initial period of 8 weeks, rats in T3 were withdrawn from beniseed extract for a period of 4 weeks. The animals were sacrificed by cervical decapitation as follows: C, T_1 and T_3 at the end of 8 th week and T_2 at the end of 12 th week.

SEMEN COLLECTION AND SEMEN ANALYSES

The caudal epididymis of one rat in each of the experimental group 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 caudal epididymis [16,17] . The semen was then taken with 1ml pipette and dropped on a clean slide and covered with cover slips. The slides were placed under light microscope and examined for morphology and sperm motility according to Saalu et al., [16,17] .

DETERMINATION OF SPERM COUNTS

The new improved hemocytometer counting chamber of Neubauers' type was used for the sperm count in accordance with Oluyemi et al., [5] . This procedure was carried out twice on each sample to eliminate error of confidence.

MEASUREMENT OF TISSUE WEIGHTS

The weights of the testes, epididymides and seminal vesicles were taken before immersing in 10% formol saline using an electrical weighing balance.

STATISTICAL ANALYSIS

Where applicable, the data obtained were analyzed statistically by students' T-test and one way analysis of variance (ANOVA). The level of significance was at <0.05.

RESULT

Figure 1

Figure 1.0 Effects of extract administration on Mean body weight of animals

Weight	Control (g)	T1 (g)	T2 (g)	T3 (g)
Initial	217±16.3	232.8±13.1	203.3±26.6	196.6±24.0
After 4 weeks Weight gained	233.2±15.5 >16±0.8	235.7±13.1 >2.9±0.0	206.6±25.9 >3.3±0.7	213.7±22.6 >17.1±1.4
After 8 weeks Weight gained	265.7±14.7 >32.5±0.8	251.2±12.4 >15.5±0.7	239.6±26.6 >33.0±0.7	221.9±22.3 >8.2±0.3
After 12 week Weight gained	-	-	251.3±21.2 >11.7±5.4	-

n = 5; Significantly different from control (P<0.05); >positive weight gain. Values are recorded as Mean±S.E.M

Figure 2

Figure 1.1 Effects of extract administration on mean weight of testes of the animals after the experiment

	CONROL♥	$T_{1(a)}$♥	$T_{2(a)}$♣	$T_{3(a)}$♥
Testis	2.19±0.15	2.30±0.30#	2.29±0.09#	2.62±0.11*
Epididymis	0.41±0.02	0.54±0.01*	0.42±0.01#	0.82±0.02*
Seminal vesicle	0.0108±0.002	0.0164±0.003*	0.0132±0.003*	0.0170±0.003*

n = 5; * Significantly different from control (P<0.05); # Not significantly different from control (P<0.05); Values measured as Mean ± S.E.M
♥: after 8 weeks; ♣: after 12 weeks

Figure 3

Figure 1.2 Effects of extract administration on the sperm count, motility and morphology

	CONTROL♥	T_1♥	T_2♣	T_3♥
Sperm Count (10^6/ml)	23.2 ± 8.9	37.6 ± 11.4	34.4 ± 11.5	41.6 ± 6.5
Sperm Motility (%)	64 ± 6.8	76 ± 13.3*	72 ± 11.2*	79 ± 3.7*
Abnormality (%)	14.6 ± 1.8	5.6 ± 7.2	5.2 ± 3.7	3.4 ± 1.7

n = 5; *Significantly higher than control (P<0.05); ᵘ Significantly lower than control (P<0.05); Values measured as Mean ± S.E.M
♥: after 8 weeks; ♣: after 12 weeks

DISCUSSION

The effects of ethanolic extract of beniseed (sesame) at 3000mg/kg body weight, with vitamin C administered as adjuvant, have shown that beniseed has a potential to increase mean body weights of rats as shown in Figure 1.0. This is most due to the high fat contents of the seed. Fats are stored in the form of Triacylglycerol (TAG) in adipose tissues in mammals via lipogenetic pathways [18]. The increased weight gain is in line with the work of [19] Shittu et al., who also recorded a dose-dependent increase in weight gain upon administration of 14.0mg/kg and 28mg/kg body weight of aqueous extract of sesame to rats for six weeks. Beniseed contains 43.0 - 56.8 % mass of fat and about 21.6 - 25.3 %mass of carbohydrate [20]. Carbohydrates are also converted to TAG through lipogenesis in the liver. Insulin increases the activity of acetylCoA carboxylase and provides glycerol for esterification of fatty acids to TAG. One of the most important carbohydrates concerned with fertility is cholesterol from which male steroids hormone (testosterone in humans or androstenedione in animals) are synthesized in the leydig cells under the influence of LH (also called Interstitial cell stimulating hormone) [18]. Thus, increased testosterone level is responsible for the increased sperm counts noted in the treated groups when compared with the control (Table 1.2). This is because FSH binds to sertoli cells and promotes the synthesis of androgen binding protein (ABP). Thus high concentration of androgen is made available locally at the seminiferous tubule, at the site of spermatogenesis.

Seminar vesicle secretes fructose (1.5-6.5mg/mL), Phosphorylcholine, Ergothioneine, Ascorbic acid, Flavins, Prostaglandins (fatty acid derivative) [21]. These chemical components of seminar fluid, coupled with the antioxidant property of Vitamin C, are responsible for enhancing motility of sperm; hence its increased secretion by the organ will lead to increased motility. In Table 1.2, there was a significant increase in the treatment group that received Vitamin C (i.e T_3). In the absence of Vitamin C, decreased motilities were recorded in treatment groups even upon withdrawal from extract when compared with the group that received Vitamin C alongside extract. In the overall, there were increases in the motility in the treatment group in a dose-dependent manner. It is also shown that ethanolic extract of sesame enhances morphology of the spermatozoa in the epididymis at the time of sacrifice of the animals. This is in agreement of the work of Saalu et al., [7].

Increased cellular activities must have led to enlargement of the component cells and invariably hypertrophy of the organs (Table 1.1). There were dose-dependent increments in the weights of selected organs; but these differences are not totally significant for all treated groups. This is a positive indication that Sesame enhances fertility by production of substances necessary for the proper function of spermatozoa. This observation is also in agreement with the work of Shittu et al [19]. Increased cellular activities are key factor to be considered in the evaluation of organ weights. Vasudevan & Sreekumari, [18] stated that androgens stimulate spermatogenesis, produce hypertrophy of prostate, seminal vesicles, muscle, bone and kidney cells. It is anabolic. This finding perfectly explains the increase weights of these organs and body weight as a whole as seen in the present experiment.

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