EFFECTS OF SANDFISH (*Scincus scincus*) ON TESTOSTERONE SECRETION AND SPERM QUALITY

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Abstract.- *The main objective of this study is to examine the effect of sandfish (*Scincus scincus*) on testosterone secretion, abundance and sperm quality. Twenty adult male rats whose body weight ranges from 150 to 210 g were used in this study. The rats were divided into four groups, the Control group (C) skink body (SB), skink head (SH) and the group who received fertility drugs (D). Rats' testosterone level, abundance, motility of spermatozoa and testicular histology were used as indices to their sexual function. The obtained results showed an increase in the rats' body weight, relative weight of the testes and testosterone level, 4.40 and 4.42 ng / ml for the SB and SH respectively compared to the C group. Similarly, the results revealed a high abundance of spermatozoa in the lumen of the seminiferous tubes and an increase in concentration ($235.37x10^6$ / ml) and sperm motility (62%) of the SB and SH rats. The obtained results indicated that* Scincus scincus *is a natural aphrodisiac with a great nutritional value.*

Keywords: *Male infertility, Motility, Nutrition, Sandfish, Spermatozoa*

EFFETS DU POISSON DE SABLE (Scincus scincus) SUR LA SÉCRÉTION DE TESTOSTÉRONE ET LA QUALITÉ DU SPERME

Résumé.- *L'objectif principal de l'étude est d'examiner l'effet du poisson de sable (*Scincus scincus*) sur la sécrétion de la testostérone, l'abondance et la qualité du sperme. Vingt rats mâles adultes dont le poids corporel se situe entre 150 et 210 g ont été utilisés dans cette étude. Les rats ont été divisés en quatre groupes, groupe témoin (C), groupe alimenté par les broyats de la partie corps du scinque (SB), groupe alimenté par les broyats de la partie tête du scinque (SH) et le groupe ayant reçu des médicaments indiqués pour la fertilité (D). Le taux de la testostérone, l'abondance, la mobilité des spermatozoïdes et l'histologie testiculaire des rats ont été utilisés comme indices de leurs fonction sexuelle. Les résultats obtenus ont montré une augmentation du poids corporel, du poids relatif des testicules et du taux de la testostérone chez les rats, de 4,40 et 4,42 ng / ml pour le groupe SB et le groupe SH respectivement par rapport au groupe C. De même, les résultats ont révélé une grande abondance de spermatozoïdes dans la lumière des tubes séminifères et une augmentation de la concentration (235,37 x 106 / ml) et de la mobilité des spermatozoïdes (62%) des rats SB et SH. Les résultats obtenus indiquent que le poisson du sable est un aphrodisiaque naturel ayant une grande valeur nutritive.*

Mots-clés: *Infertilité masculine, Mobilité, Nutrition, Poisson de sable, Spermatozoïdes.*

Introduction

Infertility is a major clinical problem, affecting people medically and psychologically [1]. It can also affect their economy, peace and harmony. Sperm counts are falling and male fertility is in the decline [2]. Many studies indicate a decrease in number and quality of human male sex cells in recent years. It seems that the disturbances of the male human sexual apparatus are multiply [3].

Different environmental factors and lifestyles have been shown to contribute to male infertility. Many of these factors affect the production and maturation of spermatozoa (spermatogenesis) by causing the production of non-viable spermatozoa or by causing abnormalities that lead to genetic defects.

80% of rural populations living in developing countries rely on traditional medicine to meet their health care needs [1]. Traditional drugs are widely used in the treatment of various metabolic disease and male impotence conditions [4].

Indeed, in the Souf region (south-eastern Algeria), men suffering from infertility due to an abnormally low sperm count, mobility or malformation can benefit from the consumption of the flesh or the powder of the Sindfish. *Scincus scincus* a reptile of the family *Scincidae*. It is heavily consumed by Souf natives for its aphrodisiac properties. The common skink is one of the lizards that has a great reputation in the therapy [5]. It has been considered as one of the most effective and valuable aphrodisiac remedies [6].

The Sandfish is an excellent source of zinc; it has a content of 37mg / 100g [7]. This value covers the recommended daily intake is 14mg / d for men and 12mg / d for women [8]. Zinc deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules [9].

The main objective of this study is to test the effect of sandfish on semen quality and mal sex hormones.

1.- Materials and method

1.1.- Animals tested

In this experiment, Skinks were served as a food complement. They were hunted from different places of bright sand. The capture of Skinks was done in the morning during the summer period (April-July) with the help of some people who are specialized and accustomed to hunting.

1.2.- Sample preparation

After killing and washing the samples, we used the traditional method to dry them [10]. After drying the samples, we have separately milled the head and the body of the Skinks using a manual grinder, the obtained ground is kept in sterile flasks for later use.

1.3.- Experimental protocol

Twenty male Albino Wistar rats, weighing between 150-210g were used in this study. The rats were procured from the Pasteur institute and kept in animal's house of

Molecular and cellular biology Department, University of El Oued, Algeria. They were acclimatized for two weeks during which they were fed a standard diet [11].

The rats were kept under normal temperature, stable humidity and a 12-hour photoperiod. Their beddings were changed every two days, they were divided into four groups of five rats each. The body weight of the rats was measured daily throughout the experiment. The rats were treated for six weeks as follows (table I).

1.4.- Blood collection and preparation of tissue samples

The rats are anesthetized with chloroform (94%) and sacrificed by decapitation. The blood is collected on dry tubes and centrifuged at 3000 rpm for 15 min, the serum is recovered and stored at (-20 ° C) and later assayed for testosterone.

Sperm was taken from a small opening made at the head of the epididymis. The samples were placed in an oven at a temperature of 37 ° C for one hour. After dissection, the testes are rinsed with 9% NaCl, then weighed and fixed in formalin to prepare histological sections.

Table I.- Groups and treatment of rats

Group	**feed**	
Control (C)	The control rats received a standard diet	water *ad libitum*
skink body (SB)	The rats were fed a standard diet containing 12% of the skink flour (body part)	
skink head (SH)	the rats were fed a standard diet containing 12% of the skimmers of the skink (head part)	
Drug (D)	The rats received standard diet containing 23.64 mg / kg of a drug indicated for male infertility	

1.5.- Testosterone measurement

Testosterone was determined using an immunometric method by immunodiagnostic products (VITROS ECi / ECiQ) using the reagent cartridge and calibration samples of VITROS testosterone [12].

1.6.- Spermogram method

The spermogram is currently a basic examination to assess the characteristics of sperm. We performed the sperm test by microscopic analysis (Nikon automated microscope) [13]. To examine the sperm count, 0.1 ml sample of semen was placed in 0.9 ml of normal saline for sperm cells to swim out in a petri-dish. The was taken to a counting chamber (haemocytometer). After the sperm cells have settled on the grid, they were viewed under the microscope and they were counted in five.

To examine sperm motility, A 20-µl sperm sample was transferred to a covered glass slide and visually analyzed with a microscope. Progressivity was determined by the grading system [1].

For the anatomo-histopathological examination, we followed the classic steps of histology. For coloring, we used the technique with hematoxylin-eosin (HE) [14].

1.7.- Statistical analysis

Statistical analysis was carried out using the test T Student-Fisher. Results are significant at the cut off $P \leq 0.05$.The results are represented in the form of average ± SEM, with signaling whether the difference between the witnesses is significant or not.

2.- Results and discussions

2.1.- Results

2.1.1.- Effect of treatments on body weight, and relative testes weight

At the beginning of the experiment (week 1-2), the body weight of the experimental and control rats showed no significant difference. However, the SH group showed a significant increase during the last 4 weeks of the experiment (week 3-4) compared to the control rats. A significant increase was observed in the SB group, The D group rats showed a significant decrease (week5-6) in their body weight during the same period.

A significant increase was observed in the relative weight of the testes, in the SB, SH and D groups compared to the control group (tab. II).

Table II.- Body weight and testes relative weight in control rats, SB skink body treated rats, SH skink head treated rats and D drug treated rats for six weeks of experience.

Body weight (g)	Control	skink body (SB)	skink head (SH)	drug (D)
Week 1-2	207.17±7.56	205.40±6.64	206.40±7.93	208.31±8.19
Week 3-4	225.86±3.71	229.00±4.56***	227.91±4.22*	224.20±2.78*
Week 5-6	236.31±3.53	245.26±9.47***	238.89±5.71*	231.82±2.85***
Testes relative weight	0.0174±0.0025	0.0188±0.0006*	0.0191±0.0028**	0.0214±0.0003***

2.1.2.- Effect of treatments on reproductive parameters

An increase in testosterone was also observed in both SB and SH groups compared to the C group (tab. III).

The spermogram analysis showed a significant difference between the three groups (SB, SH and D) with respect to the C group in the concentration of the spermatozoa and its motility.

Table III.- Reproductive parameters in control rats, SB skink body-treated rats, SH skink head treated rats, and D drug treated rats for six weeks of experience

Parameters	Control	Skink body (SB)	Skink head (SH)	Drug (D)
Testosterone (ng/ml)	1.38±0.40	4.40±3.85 ***	4.42±3.53 ***	2.06±1.00*
Spermatozoids (x106/ml)	22,42± 0,67	235,37± 7.39***	44,83±2.93***	168,12±13.14***
Mobility (%)	50±2,24	62± 10,54**	60±14,64**	74,4±3,74***

Histological sections in the testis reveal normal architecture in the control rats (fig. 1). The figure shows tight seminiferous tubules and weak interstitial spaces with a light that contains spermatozoa as well as a cell succession representing a normal evolution of spermatogenesis and spermiogenesis. We easily observed the different stages of spermatogenesis that take place centripetally at the wall of the tubes. Small spermatogonia is located near the basement membrane. Spermatocytes I and II of larger sizes. The smaller spermatids were located towards the inside of the tubes.

The spermatozoa wall filled all the light of the seminiferous tubes with their flagella. In all three treatment groups, testicular histology showed an increase in sperm count in seminiferous tubule lumen, spermatozoa abundance in the SB group was higher than D group and SH compared to control.

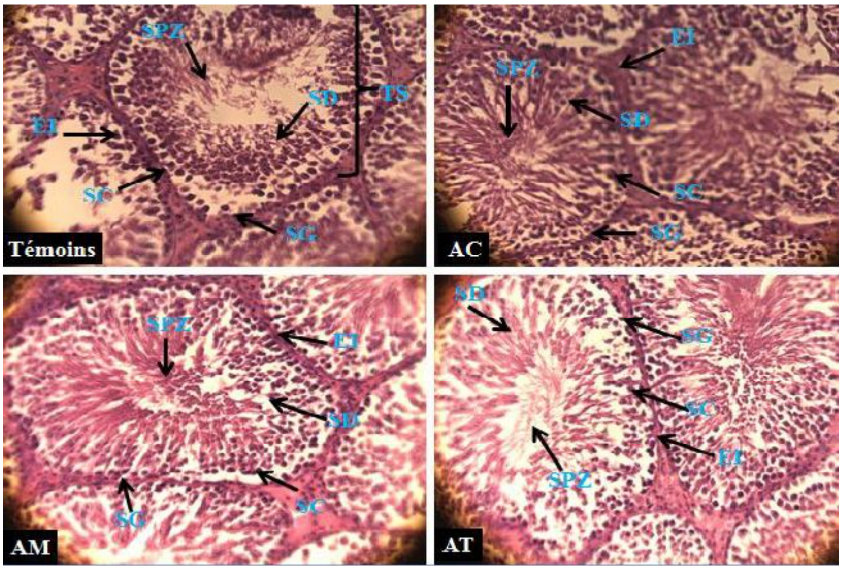

Figure 1.- Histological structure of the testes of four lots X400 (TS: Seminiferous Tubes, EI: Interstitial space, SG: Spermatogonia, SC: Spermatocytes I and II, SD: Spermatids, SPZ: Spermatozoa)

3.- Discussion

Consuming sandfish leads to weight gain in rats. This study is in line with the studies that have found that administering aqueous extract of *Hibuscus macrocanthus* and *Basella alba* causes weight gain [15]. The aphrodisiac effect of the aqueous extract of *rauvolfia obscura k. Schum* (apocynaceae) on rats, is also accompanied by a significant increase in body weight [16].

In terms of weight gain, the most basic rule is to consume more calories than you burn. The quality of this weight (mostly muscle, or mostly adipose tissue) is closely related to the sources of nutrients and calories consumed and how the muscles have had to adapt to the constraints of Physical activity [17], However, weight loss occurs when you consume less energy (kcal) than what your body uses [18].

In this study, the increase in weight of SB rats is due to the very rich composition of protein, lipid, sugar and mineral salts of *Scincus scincus* [7].

When an acceleration of testicular growth occurs, it is essentially under the effect of the establishment of spermatogenic function characterized by an increasingly intense cell multiplication and by the increase in the importance of seminiferous tubules (size and relative proportions) [19]. a reproductive organ development (increased testicular and seminal vesicle weight in males) were also seen in a previous study [20]. Also diet has a major impact on the functioning of the male gonad, dietary supplements such as micronutrients prescribed for the purpose of improving fertility [21], Zinc deficiency causes both, a reduction in testicular size [22], and disorders of sexual maturation (gonadal atrophy (testes, ovaries) [23].

The administration of micronutrients induces an increase in serum testosterone level [24,25] which is consistent with the richness of sandfish by minerals (39.50% of MS) [7]. This increase can be explained by the presence of steroidal components in the ground meat of the Skink.

Many studies have found that on aphrodisiac plants contain compounds of steroidal structure. These compounds act at the level of the hypothalamus, increasing the secretion of luteinizing hormone, LH, which leads to an increase in testosterone production [26-29]. Statistical analysis of body weight and testosterone shows a positive correlation ($r = 0.72$) between these two parameters. This indicates that an increase in rats' body weight is associated with an increase of testosterone level. Testosterone acts as both an aphrodisiac and an anabolic hormone that helps bones and skeletal muscles to grow [30].
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he effectiveness of ground fish is similar to that of the drug that stimulates spermatogenesis and increases the concentration and mobility of spermatozoa. "The ancients sold the flesh of the common skipper a lot, it has the capacity to promote an abundant secretion of sperm"[31].

Sperm concentration is a function of testosterone production following release of LH, which acts on Leydig cells and causes the secretion of testosterone, which stimulates spermatogenesis [32].

The high sperm count in rat groups treated by sandfish can also be explained by the high concentration of zinc (37 / 100g). Zinc leads to an increase in male sex hormones and sperm quality in rats [33,34]. This effect was highlighted by another study which revealed that Zinc supplementation has proved to be beneficial in the sterility of males and necessary for the formation and maturation of spermatozoa [35].

The increase in sperm motility of the treatment rats compared to that of the control group can be explained by the nutritional value of sandfish. According to previous studies that investigated the impact of nutrients (vitamins, minerals, metals or PUFAs) on the sperm quality, an omega-3 supplementation has a beneficial effect on sperm quality, especially on its mobility [36].

Conclusion

The objective of this study is to examine the effect of sandfish consumption on reproductive parameters.

The results obtained show an increase on testes relative weight, testosterone, concentration and mobility of spermatozoa, and sperm abundance at seminiferous tube level.

The obtained results show that sandfish (*Scincus scincus*) has some androgenic activity that can correct a testicular failure in the male.

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