

The Effect of Chronic Khat Consumption on Sperm Count and Motility in Parent Mice and Their Offspring

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Abstract

Khat is considered a psychoactive drug and has many side effects on different parts of the body organs. In this study the effects of khat on sperm count and motility in parent mice and their offspring were investigated. Animals were divided into two groups, Group 1 (khat group) contained twelve male and twelve female mice, and they were given a daily dose of (50mg/kg) body weight khat extract by gastric gavage for four and eight weeks. Group 2 (control group) also contained twelve male and twelve female mice and received normal access of food and water. After four weeks of treatment, the males and females were allowed to mate and khat treatment continued for up to another four weeks. Twenty four male offspring from group 1 and group 2 were selected randomly and allowed to become mature. Male parent mice were killed at the 4th and 8th weeks of treatment, and their male offspring were killed when they reached maturity age (6-8weeks). Physiological examination of the sperm solution showed that there was a significant increase in sperm count and motility after 4 and 8 weeks of khat treatment, and in their adult offspring. Furthermore, histological changes were found in testicular sections of the adult male mice.

Keywords: Khat, offspring, sperm, testes

1. Introduction

Khat "*Catha edulis*" belongs to the plant family Celastraceae and it refers to the leaves and young shoots. The habit of chewing khat has prevailed for centuries among populations (males and females) of African horn and Arabian Peninsula including Yemen. Khat contains many different compounds and therefore may have many effects on different parts of the body. The effect of khat leaves on the central nervous system is considered as the most important effect causing euphoria, increased alertness,

logorrhoea, hyperactivity, anxiety, and aggressiveness and may be followed by insomnia [1]. The effect on anxiety and depression is temporary and disappears the next day [2]. Long term chronic users may develop personality disorders and mental deterioration [3].

Although, detailed studies on the effects of khat on reproduction are limited, khat has been shown to produce reproductive toxicity in human beings and experimental animals [4]. Chronic chewing of khat may cause spermatorrhoea and may lead to decreased sexual functioning and impotence [5]. Animal models have also demonstrated that khat reduced placental blood flow and resulted in growth retardation of the offspring [6]. Even though khat has deleterious effects on many parts of the body, but its exact effect on sperm parameters have not been well investigated. None of these studies describe the effects of khat feeding during gestation on the offspring's spermatozoa. Therefore the aim of the present study was to investigate the effect of khat on sperm count and motility in parent mice and their offspring.

2. Materials and Methods

2.1. Animals

Forty eight Swiss albino mice of the BALB/c strain of both sexes weighing 25-30 g and aged 7-8 weeks were used. The mice were in good health and bred in the Animal House, College of Science, Sana'a University and were kept at room temperature 20-24 C^o, they had free access to laboratory chow and water.

2.2. Khat Leaves

Leaves of khat were obtained from a crop in Haja (Sawty type) not sprayed with insecticides. The young leaves and shoots of khat were washed with distilled water and dried at room temperature of 24 C^o in the laboratory. The dried leaves were powdered in an electrical grinder and the powder was kept in plastic bags in the freezer at -20 C^o until its usage.

2.3. Preparation of Khat Methanolic Extract

The methanolic extract of khat was prepared by extracting 20 g of the powdered khat using methanol as a solvent by the soxhlet apparatus, the solution was subjected to a rotary evaporator under pressure and 45 C^o for 45 minutes. The yellowish-brown crude extract was then kept in an incubator till it dry and finally the dried crude extract was yielded, weighed and kept in a dark bottle in the refrigerator until used in the experiment.

2.4. Experimental Design

Twenty four experimental (khat "K" group) and twenty four control (C group) mice were used in this experiment. Each of the khat group contained 12 male and 12 female animals. The "C" control group was also of 12 male and 12 female animals. The khat "K" group received daily a dose of 50 mg/kg khat extract orally [7], for four and eight weeks respectively. After four weeks of treatment, the males and females were allowed to mate and khat treatment continued for up to another four weeks and stopped. Twelve male offspring obtained from the khat group and twelve male offspring from the control group were selected randomly and allowed to reach maturity age (6-8weeks). Male parent mice were killed at the 4th and 8th weeks of treatment, and their male offspring were killed when they reached maturity age.

2.5. Histological Examination

The removed testes were fixed with Bouin's solution for 48 hr, dehydrated by series of ethanol, cleared in xylene and impregnated and embedded in paraffin wax. Paraffin sections of 5 μ m thickness were stained by with haematoxylin and eosin.

2.6. Sperm Count & Sperm Motility

Immediately after killing the animals; the caudal epididymis of each mouse was removed; placed in a small clean Petri containing 1ml of Phosphate Buffer Saline (pH=7.4), cut by a sterilized blade into three pieces and squeezed by a fine forceps in order to release the sperm and then left at 37 C^o for 10 minutes [8]. Sperm were counted using a Neubaur Hemocytometer Chamber under the microscope at 100X magnification [9]. Sperm motility was determined by using the same sample of sperm solution in the Hemocytometer chamber and the number of motile sperm was counted as percentage of sperm motility in the central counting area [10]. Sperm count and motility was performed four times for each sample [11].

2.7. Statistical Analysis

Statistical analysis was performed using SPSS Statistical Program. Results were analysed statistically using Student's t-test and paired samples t-test to compare the significance of the differences between the means of tests and control studies. The results were expressed as mean \pm standard deviation. *P-values* less than 0.05 were considered to be significant and less than 0.01 as highly significant.

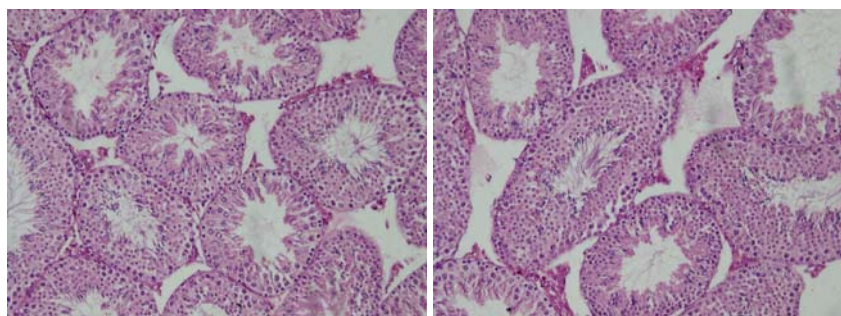
3. Results

After eight weeks of treatment with khat, testes of parent mice showed an increased tendency of spermatogenic process with high density of spermatozoa in seminiferous tubules (Figure 1 "A"). The testes of the offspring showed regular epithelial lining of the seminiferous tubules and some had accumulations of sperm, in the lumens (Figure 1 "B").

Khat feeding showed a high significant increase ($P<0.01$) in the parent spermatozoa number (148 ± 8.41) 10^6 /ml at the 4th week compared to the control mice (76.33 ± 9.03) 10^6 /ml and (158 ± 8.94) 10^6 /ml at the 8th week compared to the control mice (70.33 ± 9.41) 10^6 /ml. The offspring of the parent mice also showed a significant increase ($P<0.05$) of the spermatozoa number (85.83 ± 8.93) 10^6 /ml compared to the control (67.33 ± 10.54) 10^6 /ml (Table 1).

A highly significant increase ($P<0.01$) in sperm motility of parent mice (106.33 ± 8.14)% compared to control mice (52.5 ± 4.04)% at the 4th week and (124.83 ± 12.18)% at the 8th week compared to the control mice (46.67 ± 6.71)% was observed. The offspring also showed a significant increase ($P<0.05$) in sperm motility (58.83 ± 5.64)% compared to the control (48.33 ± 7.26)% (Table 1).

Figure 1: Testes of the khat group (A) after eight weeks of treatment, and of the offspring of the khat group (B) showing normal structure of seminiferous tubules filed with spermatocytes, spermatids and spermatozoa .(H&E).100X



A

B

Table 1: Average of sperm count and sperm motility of parent mice treated with khat and their offspring expressed as (Mean ±Sd).

	Parental Mice				Offspring Mice	
	C1 (n=12)	K1 (n=12)	C2 (n=12)	K2 (n=12)	C3 (n=12)	K3 (n=12)
Sperm count (10⁶/ml)	76.33 ±9.03	148 ±8.41**	70.33 ±9.41	158 ±8.94**	67.33 ±10.54	85.83 ±8.93*
Sperm motility (%)	52.5 ±4.04	106.33 ±8.14**	46.67 ±6.71	124.83 ±12.18**	48.33 ±7.26	58.83 ±5.64*

Values are expressed as Mean±Sd

* Significant (P<0.05) to their control.

** Highly Significant (P<0.01) to their control.

C1: control group (Parent mice allowed for free food for 4 weeks).

K1: khat group (Parent mice treated with khat for 4 weeks).

C2: control group (Parent mice allowed for free food for 8 weeks).

K2: khat group (Parent mice treated with khat for 8 weeks).

C3: control group (offspring of parent mice allowed for free food).

K3: khat group (offspring of parent mice treated with khat).

4. Discussion

The present study illustrates the effects of khat leave extract on the histological and some physiological (sperm count and motility) properties of the testes and sperm in parent mice and their offspring. Detailed studies on the effects of khat on reproduction are limited; however, the limited available data reveal that chewing of khat has an impact on human and animal's reproductive systems. Islam *et al.* [4] observed degeneration of interstitial tissue, cellular infiltration and atrophy of Sertoli and Leydig cells associated with a significant decrease in plasma testosterone levels in khat treated rats, while histological examination of sections from the male reproductive tract of rabbits showed that khat leaves had increased spermatogenesis and Leydig cells were unaffected when compared with untreated rabbits [12].

It is well known that the major function of the testes is spermatogenesis and hormone synthesis. Sperm count is an indicator of spermatogenesis and sperm motility is an indicator of fertility [13]. In the present study, a significant increase in the sperm count and motility in both the parent mice and their offspring was observed in comparison with control animals. This is in accordance with Al-Mamary *et al.* [12] who observed an increase in the rate of spermatogenesis along with spermatozoa in male rabbits fed on khat leaves. In addition, Mekasha *et al.* [14] detected similar results and found that supplementation with khat leftovers induced the greatest improvement in live body weight, testicular size, semen production and sperm motility in Ogaden goats. However, other studies have shown that heavy use of khat causes decreased semen volume, sperm count, sperm motility, and increased number of abnormal sperm [15,16], and degeneration of interstitial tissue, cellular infiltration and atrophy of Sertoli and Leydig cells [4]. The results of this study reveal that a continuous administration of khat extract for eight weeks induces an increase in sperm count and motility by stimulating spermatogenesis. These differences may be attributed to the dose, duration of consumption and whether the plant contains or free of pesticides or other pollutants. Pesticide exposure induced disruption to seminiferous tubules and may affect spermatogenesis leading to poor semen quality and reduced male fertility [17]. Pollutant factors such as lead could negatively affect the spermatogenesis [13,18]. The present administered khat was 100% free of pollutant.

It has been confirmed that an enzyme called GAPDS (glyceraldehyde 3-phosphate dehydrogenase-S) exists in mice sperm and it is essential for sperm's motility and ATP production

[19]. Recent studies by Adeoya-Osiguwa and Fraser [20], have shown for the first time that PPAs (phenylpropanolamines) which is formed by cathine and norephedrine chemicals in khat stimulate the production of CAMP (cyclic-adenosine monophosphate) which stimulate the sperm motility at the final stage of maturation.

Recently Bedada and Engidawork [21], indicated that khat exposure produces dose-related central and peripheral effects during pregnancy and lactation which might cause a serious impediment to the physical and mental development of the offspring as well as alterations in the biochemical indices of liver and kidney functions. The present study showed a significant increase in both sperm count and motility of mice offspring and it is possible that khat affected their mothers during pregnancy [22]. The mechanism by which khat affects the testes of the offspring have not been elucidated yet. It has been demonstrated that lead was able to transfer across the human placenta around the 12th to 14th week of gestation [23] and in rats [24] and mice [18]. It is possible that the major components of khat are capable to reach the embryonic tissues at different periods of gestation in the mouse; however further biochemical studies are needed to detect khat's major components in the embryo and offspring.

5. Conclusion

Khat extract at a dose of 50mg/kg lead to increased tendency of spermatogenic process, as well as, it causes a marked increase in both sperm count and motility in parent mice and their offspring.

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