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The potential adverse effects of khat chewing and cigarette smoking on human sperm parameters

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Abstract

The combined effect of khat chewing and cigarette smoking is confusing and no standard general guideline documented**.** The current study aims to evaluate the potential adverse effects of khat chewing and cigarette smoking on human sperm parameters of patients. Semen samples were collected from 507 patients of couples consulting for infertility recruited in this study and divided into three groups; (i) non-smokers and non-khat chewers group (n=77), (ii) smokers group (n=142) and (iii) smokers and khat chewers group (n=288). Seminal volume, sperm count, motility, vitality, and normal morphology were evaluated according to guidelines of World Health Organization (WHO). A highly significant differences ($p<0.0001$) were recognized between the three groups in volume (ml) of semen and the percentage of count, motility, vitality and normal morphology of the sperms. Moreover, semen volume (ml) was significantly ($p<0.010$) connected with sperm motility (%) ($r=0.100$) and normal morphology (%) ($r=0.168$), also, sperm count (mill/ml) was significantly ($p<0.010$) associated with sperm motility (%) ($r=0.379$), normal morphology (%) ($r=0.386$) and vitality (%) ($r=0.244$). In addition, sperm motility (%) was highly and significantly ($p=0.0001$) correlated with sperm normal morphology (%) and vitality (%) ($r=0.446$; $r= 0.284$, respectively). A significant ($p=0.000$) correlation also conducted between sperms with normal morphology (%) and sperm vitality (%) ($r=0.225$). These findings suggest that chewing khat and cigarette smoking may be a source of high oxidative stress and accordingly deteriorate semen quality.

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Introduction

Khat (*Edulis cathis*) is a plant belongs to the family Celastraceae. It contains over 40 different chemical compounds like amino acids, vitamins, alkaloids, tannins, glycosides, and minerals. Cathinone and cathine are phenylalkylamines contained in khat are responsible for the effect of chewing khat. They are a non-narcotic bioactive compound with amphetamine like effect on nervous system (Halbach, 1972). People whom chewing khat looking for its stimulant effects, euphoria, feeling of well being and excitement (Tariq, *et al.*, 1987). Khat was considered by world health organization (WHO) in 1964 as a material that can cause psychological addiction (Clement, *et al.*, 2012). Studies on mice found that cathine stimulate the synthesis of cAMP in uncapacitated sperm suspensions, but significantly repressed it in capacitated suspensions (Adeoya-Osiguwa and Fraser, 2005), it acts on mammalian spermatozoa via beta1- and alpha2A-adrenergic receptors in a capacitation state-dependent manner (Adeoya-Osiguwa and Fraser, 2007). Khat is planting in East part of Africa (Mekasha *et al.,* 2007) and in the South of the Arabic area (Nyongesa, *et al.*, 2008). In addition, In rats, a decline in sperm concentration, motility and testosterone level in plasma, higher levels of abnormal sperms, degeneration of Leydig and Sertoli cells were detected (Islam, *et al.*, 1990); (Tariq, *et al.*, 1990). Khat also had harmful effects on semen parameters in general and particularly morphology of sperm (el-Shoura, *et al.,* 1995). A decreased in sperm parameters (count, motility and morphology) and semen volume, were revealed in khat chewers (Hakim, 2002). Furthermore, Also, khat found to elevate the oxidative stress. High levels of plasma lipid peroxidation biomarker malondialdehyde, (MDA) were detected in both groups' khat chewers and in khat chewers and smokers (Al-Zubairi, *et al.*, 2003).

Not like reports mentioned above that suggesting deleterious effects on the male reproductive tract, opposite results found by two studies: the first illustrated improvement of Ogaden bucks body mass, testes size, semen production and motility and morphology of sperms (Mekasha, *et al.*, 2007, Mekasha, *et al.*, 2008). The second study by conducted by (AlBadri, *et al.*, 2012), concluded that khat extract at a dose of 50mg/kg leaded to increased tendency of spermatogenic process, as well as, it increased both sperm count and motility in parent mice and their offspring.

Besides, cigarette smoke was found to be a significant risk factor that might affect male fertility (Vine, 1996). As is recognized, cigarette smoke contains 4000 chemical substances, out of which about 55 are carcinogenic and around 400 toxic (Kumar, *et al.*, 2011). The high concentrations of the free radicals were detected and it may induce the production of cellular reactive oxygen species (ROS) in the human body (Zang, *et al.*, 1995, Pryor, *et al.*, 1998). Nicotine is main powerful oxidizing agent in cigarette compound that disturbs the DNA integrity and plasma membrane of the sperm (Arabi, 2004).

Numerous studies revealed that cigarette smoking is associated with bad sperm quality like low concentration, motility, and morphology, and altered sperm function (Hammadeh, *et al.*, 2010) as well as decrease the outcomes of assisted reproductive therapies (ART) (Soares and Melo, 2008) (Anderson, *et al.*, 2010).

On the other hand, some studies concluded that smoking cigarette and drinking alcohol do not appear to pointedly affect sperm parameters; sperm count, motility and morphology or semen volume, and pregnancy outcomes of the studied populations (Ozgur, *et al.*, 2005) (Jong, *et al.*, 2012).

Reactive oxygen species (ROS) are molecules that carry one or more unpaired electrons; therefore, they are very reactive, specifically to lipids. As plasma membrane of sperm is rich in polyunsaturated fatty acids (PUFA); particularly long-chain PUFA docosahexaenoic acid and docosapentaenoic acid that make it sensitive to lipid peroxidation particularly by ROS (Brinsko, *et al.*, 2005). ROS high concentrations were detected in seminal plasma of infertile smokers

(Saleh, *et al.*, 2002, Colagar, *et al.*, 2007, Kiziler, *et al.*, 2007, Soares and Melo, 2008, Hammadeh, *et al.*, 2010). Even though, low levels of ROS are required for acrosomal integrity, capacitation, and hyperactivation of the sperm and also for the fusion of sperm and oocyte (Awda, *et al.*, 2009). In addition, when ROS levels overcome antioxidants levels in the body, oxidative stress occurs, that also may result in oxidative damage to DNA. However, the mechanisms of cigarette smoke–associated damage to human spermatogenesis are still largely unknown.

The compensation of the cigarette smoking and khat chewing effects, though, was not thoroughly studied. Mostly cigarette smoking and khat chewing are habitually seen together, this study emphases on the outcomes of smoking and khat chewing lifestyles separately and combined on semen characteristics, such as seminal volume and count, motility, vitality and morphology of the sperm. These proposed adverse effects are evaluated in three groups of patients recruited in this study; non-smoker and non-khat chewer group, smoker group and smoker and khat chewer group.

Materials and methods

Semen Parameters in This Study

This study evaluated the semen parameters: seminal volume, sperm count, sperm motility, sperm vitality, and valuation of sperm morphology following the 2010 WHO criteria.

Study population

Samples of semen were obtained from male partners (n=507 patients) of couples accessing for infertility at IVF lab in Al-UM Hospital in Yemen. The patients were divided into three groups: (i) non-smokers and non-khat chewers (n=77), randomly selected from the patients, (ii) smokers (n=142), smoke more than 20 cigarettes per day and (iii) smokers and khat chewers (n=288), daily chew khat and smoke. Information about body height, weight, and smoking, drinking of alcohol and working exposures was obtained from a questionnaire.

All reagents that used in this research bought from Sigma, Germany unless other companies stated.

Semen Analysis

Ejaculates were collected in sterile containers by masturbation after 3 to 7 days of sexual limitation. A single sample of each patient was used in the study. After liquefaction of samples for 30 minutes in 5% CO2 incubator at 37^{o}C. Analyses of semen volume, pH, sperm count, motility and vitality were assayed for within 2 hours after collection. A standard semen analysis was performed according to WHO criteria Basic sperm characteristics; concentration, and motility were evaluated by using Mackler counting chamber (Sefi Medical Instruments Ltd.).

Assessment of Sperm Vitality (Eosin Test)

On a glass slide, 5 µl of liquefied seminal fluid was mixed with 5 µl of 0.5% aqueous yellow eosin Y solution. The mixture covered with a cover slip, then evaluated within 2 minutes by distinguishing between the dead sperm (Red and the live sperm (white). 200 sperms were assessed from each slide under light microscopy with oil immersion lens with magnification power of X 1,000.

Assessment of Sperm Morphology

Semen smears were prepared by spreading 10 µl of semen on a glass slide and stained using Papnucleou method. 100 sperms from each slide were counted using oil immersion lens at a magnification power of 1000 X by bright field illumination. At least 10 fields from various areas of the slide were examined (World Health Organization, 2010). Sperm morphology of evaluated sperms was distinguished as morphologically normal or abnormal.

Statistical Analysis

Kolmogorov–Smirnov test was used to assess if the data were normally distributed or not. The relationships between conventional sperm parameters (volume, count, motility, viability and morphology) were analyzed by nonparametric methods. Statistical analysis was done using Sperman's correlation test and the non-parametric

Kruskal-Wallis test and also used to assess differences between the samples from the three groups; non-smoking and non-khat chewers patients, smoking patients and smoking and khat chewers patients, where a probability value of $p<0.050$ was considered significant and $p<0.010$ was considered highly significant. The statistical analyses were performed out by the SPSS 19 for Windows Software Package (SPSS Inc., Chicago, IL, USA).

Results

The semen sample involved in the present study (n=507) were distributed into three groups: 77 Non-Smokers and non-Khat Chewers (mean age: 36.75±5.27), 142 smokers (mean age: 35.00±6.18) and 288 Smokers and Khat Chewers (mean age: 35.11±7.99) and there was no significant ($p>0.05$) different between the three (Table 1).

Table 1. Sperm and seminal plasma parameters of all patient groups.

Parameters	All (n=507)	Non-Smokers and Non-Khat Chewers (n=77)	Smokers (n=142)	Smokers and Khat Chewers (n=288)	p-value
Age (year)	35.33±7.16	36.75±5.27	35.00±6.18	35.11±7.99	0.134
Volume (ml)	2.51±1.44	2.86±1.67	2.92±1.46	2.22±1029	0.000
Count (mill/ml)	50.40±33.61	69.77±31.07	55.11±31.97	42.90±32.65	0.000
Motility (% motile)	29.19±16.31	34.22±18.27	26.46±15.16	29.19±16.04	0.000
Morphology (% normal)	7.18±4.20	10.67±4.15	7.51±3.14	6.09±4.17	0.000
Sperm vitality (Eosin Test) (%)	37.26±14.18	41.88±18.21	38.19±17.08	35.57±10.70	0.000

P<0.001 Highly significant.

Conventional sperm parameters

Analysis of semen samples was done for all patients according to WHO guidelines. A highly significant differences ($p<0.0001$) were established between the three groups (non-smokers and non-khat chewers group, smokers group and smokers and khat chewers group) in sperm concentrations (%) (69.77±31.07; 55.11±31.97; 42.90±32.65, respectively) and motility (%) (34.22±18.27; 26.46±15.16; 29.19±16.04, respectively). In addition, the mean numbers of morphologically normal sperm, and vitality were significantly lower ($p<0.0001$) in smokers and khat chewers group (6.09±4.17%; 35.57±10.70%) than smokers group (7.51±3.14%; 38.19±17.08%) in comparison to that of non-smokers and non-khat chewers (10.67±4.15%; 41.88±18.21%) (Table 1, Fig. 1). A significant differences ($p<0.0001$) were verified between all groups in semen volume (ml) (Table 1).

Correlations between sperm parameters

The Spearman's correlation was applied to study the correlations between sperm parameters of all groups. In table 2, statistical significant ($p=0.000$) correlations were detected between the sperm concentrations (10^6/ml) and sperm motility (%) ($r=0.379$), normal morphology (%) ($r=0.386$), and vitality (%) ($r=0.244$). Also, sperm motility was highly significant correlated with normal morphology ($r=0.446$, $p=0.000$), and vitality (%) ($r=0.284$, $p=0.000$). Similarly, morphologically normal sperm was highly and positively ($p=0.000$) correlated with sperm vitality ($r=0.225$). In addition, semen volume (ml) was significantly ($p<0.050$) correlated with sperm motility, and morphology and non-significantly ($p>0.050$) with sperm count and vitality.

Disscusion

This study conducted on a heterogeneous population. It demonstrates the significant adverse effects of chewing khat and smoking cigarette on sperm

parameters (count, motility, morphology, and vitality) in comparison with non-smoking and non-khat chewers (control group).

Cigarette smoke is considered as one of the risk factors for male infertility (Vine, 1996).

The impact of cigarette smoking on standard sperm parameters and male fertility was highly investigated by many research groups. (Künzle, *et al.*, 2003) showed a harmful effect of smoking on sperm parameters. Also, (Pasqualotto, *et al.*, 2006) showed that semen volume smokers was lower than that of non-smokers, and this drop in semen volume was correlated to the number of smoked cigarettes and this is in agreement with results of this work (Table 1). A significant high teratozoospermia and reduction in sperm motility were illustrated in heavy smokers likened to that of non-smokers (Gaur, *et al.*, 2007). In addition, a significant reduction in sperm parameters (count, motility, vitality and morphology) was established by (Hammadeh, *et al.*, 2010). Recently, (Yu, *et al.*, 2014) calculated that both smoking and altered semen quality were strongly connected with the histone-to-protamine transition in mature human sperm.

Table 2. Correlations of sperm and seminal plasma parameters of All patient (n=507).

		Age (year)	Volume (ml)	Count (mill/ml)	Motility (% motile)	Morphology (% normal)	Sperm vitality (Eosin) (% vital)
Age (year)	R	1.000	-.116-**	-.031-	-.070-	-.070-	-.108-*
	P	.	.009	.480	.118	.115	.015
Volume (ml)	R	-.116-**	1.000	.085	.100*	.168**	.079
	P	.009	.	.055	.024	.000	.074
Count (mill/ml)	R	-.031-	.085	1.000	.379**	.386**	.244**
	P	.480	.055	.	.000	.000	.000
Motility (% motile)	R	-.070-	.100*	.379**	1.000	.446**	.284**
	P	.118	.024	.000	.	.000	.000
Morphology (% normal)	R	-.070-	.168**	.386**	.446**	1.000	.225**
	P	.115	.000	.000	.000	.	.000
Sperm vitality (Eosin) (% vital)	R	-.108-*	.079	.244**	.284**	.225**	1.000
	P	.015	.074	.000	.000	.000	.

P< 0.05 Significant.

P< 0.001 Highly significant.

Cigarette smoking strongly correlated with high ROS levels in seminal plasma that result oxidative stress (Saleh, *et al.*, 2002). (Pasqualotto, *et al.*, 2008) illustrated that cigarette smoking may negatively associated with poor sperm quality and decreases the antioxidant capacity in seminal plasma. Smoking may prompt variations in the plasma membrane of sperm as it contains high amount of PUSFA and also a high levels of DNA damage (Church and Pryor, 1985). Number of studies reported high significant levels of DNA damage in smokers sperms than those of non-smokers (Sepaniak, *et al.*, 2006) (Elshal, *et al.*, 2009) (Hammadeh, *et al.*, 2010). Altogether, sperm histone transition could be affected by cigarette smoking at the level of protamine mRNA transcription.

The present study illustrated the deleterious effect of smoking cigarette. Sperm parameters; count, motility, vitality and morphology and semen volume were significantly lower ($p<0.001$) in smokers than that of non-smokers and non-khat group (control group). These results are in accordance with previous works done by (Künzle, *et al.*, 2003, Pasqualotto, *et al.*, 2006, Gaur, *et al.*, 2007, Pasqualotto, *et al.*, 2008, Hammadeh, *et al.*, 2010, Yu, *et al.*, 2014).

On the contrary, number of studies did not find association or adverse effects from smoking cigarette and drinking alcohol on semen parameters (Shen, *et al.*, 1997) (Okonofua, *et al.*, 2005) (Ozgur, *et al.*, 2005). Moreover, (Jong, *et al.*, 2012) showed that

smoking cigarette and drinking alcohol did not significantly affect sperm parameters, like sperm count, motility and morphology and volume of semen volume, or pregnancy outcome.

Comprehensive studies on the khat effects on reproduction are limited; however, the limited available data revealed that chewing khat is a risk factor on human and animal reproductive systems.

(Hakim, 2002) revealed a decrease in semen volume, sperm count, motility and morphological alterations in khat chewers compared to non-chewers, even though no significances between changes were detected.

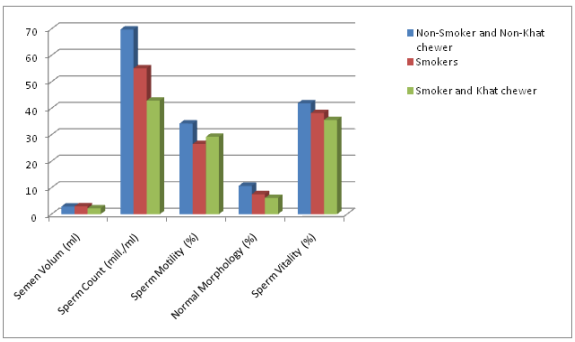

Fig. 1. Comparison between semen volume (ml) and sperm parameters (count, motility, morphology and vitality) in Non-Smoker Non-Khat Chewers group, Smokers group and Smokers and Khat Chewers group patients. Significant differences ($p<0.001$) were found for all parameters between all groups).

Besides, in animal studies; (Islam, *et al.*, 1990) and (Tariq, *et al.*, 1990) showed drops down in sperm concentration and motility by 28 and 22%, respectively, a 1.53-times increase in the level of altered sperm, and a 50% decline in plasma testosterone from khat supplementation (cathinone) in rats. Nevertheless, number of reports presented a mixture of negative and positive effects from khat extract on sex hormones concentrations, sperm action and morphology. Also, in mice it was found that speed up of sperm capacitation and inhibition of spontaneous acrosome loss of sperm might happen at certain doses of khat constituents (cathine and norepherdrine) (Adeoya-Osiguwa and Fraser, 2005, Adeoya-Osiguwa and Fraser, 2007). In another study conducted on mice also by (Nyongesa, *et al.*, 2007), they found that testosterone production was inhibited with high levels of khat feeding while stimulation occurred with low levels of khat. Similarly, in male rabbits supplemented with khat extract testosterone and luteinizing hormone levels were decreased in a dose–response case (Nyongesa, *et al.*, 2008).

The current study revealed that significant declines in sperm count, motility, vitality and normal morphology and semen volume in khat chewers and smokers group than the group of smokers only and non-smokers and non-khat chewers group. These results are in convenient with that of (Islam, *et al.*, 1990), (Tariq, *et al.*, 1990), (Hakim, 2002).
The pharmacological effects of chewing khat are resulted mainly from its constituent cathinone that look like amphetamine in biological effect and chemical structure (Kalix and Braenden, 1985).

Even so, a confliction is found by a study carried by (AlBadri, *et al.*, 2012) who showed significant increases in both sperm concentration and motility of mice offspring. The mechanism by which khat affects testes of the offspring have not been clarified yet.

The mutual effect of cigarette smoking and chewing khat was not thoroughly inspected and no generally conventional guideline recognized (Okonofua, *et al.*, 2005) (Anderson, *et al.*, 2010). However, the relationship between khat chewing and cigarette smoking is confusing as noted by (Amha, 1983) and (Zein, 1988). This study clarified the adverse combined effects of khat chewing and cigarette smoking on sperm parameters. All sperm parameters of the smokers and khat chewers found to be significantly ($p<0.001$) lower than that of smokers group compared to that of control group (Table 1, Fig. 1).

One study once, presented that consumption of khat with cigarette, coffee and or alcohol combination was widespread through nations, social levels and age groups. Also demonstrated that khat has detrimental effects on seminal fluid features (Hakim, 2002).

Though, semen quality may depend on the ejaculate, subject physiological conditions, or other factors.
In addition, the mutagenic possible effect of khat was studied in rats and resulted in low levels of nucleic acids in brain and liver and chromosomal abnormalities in bone marrow (Dehondt, *et al.*, 1984). Moreover, spermatogenesis process may be deteriorated in khat chewers as a result of long sitting in doubled up position and the hypothermia which is a feature of khat chewing. Furthermore, the inverse effect of cigarette smoking and khat chewing on semen quality may be due to elevation of ROS concentrations that resulted from long period of smoking and khat consumption. Alterations in sex hormones also observed in khat chewers may be another cause of the inverse effects of khat on semen quality. It may well be that khat chewing has an self-determining dose-related risk factor for the development of deleterious effects on semen quality but because khat chewers are almost smokers, it is, therefore, hard to separate khat as an independent risk factor for male fertility and, hereafter, verification requires further experimental work.

Moreover, the negative effects of khat consumption on sperm quality may be resulted from the pesticides or other pollutants that absorbed by khat and enter the body when consuming khat. Pesticides found to prompt a damage of seminiferous tubules that negatively affect spermatogenesis resulting in poor semen quality and reduced male fertility (Roeleveld and Bretveld, 2008). Also, presence of other pollutants like lead may adversely affect spermatogenesis process (Al-Ani, *et al.*, 2009, Al-Khfaji, *et al.*, 2011).

In conclusion, the current study demonstrates that both smoking and khat chewing are strongly associated with defective semen quality and may be a reason of infertility. However further biochemical studies are needed to detect the molecular mechanism through which khat's major components affect semen quality.

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