Effect of Khat chewing and Seminal Oxidative Stress on Male Infertility

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Abstract

Background: Infertility is one of the most important problems that despairs couples in their social life. Globally, it impacts millions of people of reproductive age, including men and women. In general, male factor infertility takes part in approximately 50% of all cases of infertility. Oxidative stress may be implicated in 30–80% of male infertility etiology due to damaging sperm DNA and impairing sperm quality and function. According to the literature data, Khat consumption increases oxidative stress, however, showed a discrepant effect on sperm quality. In this light, this study was conducted to assess and compare the levels of seminal ROS and TAC among 100 Yemeni men classified into infertile Khat chewers (25) and infertile non-Khat chewers (25) as case groups and fertile Khat chewers (25) and fertile non-Khat chewers (25) as control groups. It also aimed to evaluate the oxidative stress effect along with the Khat effect on sperm quality.

Results: We found that MDA concentration was significantly higher in infertile non-Khat chewers and infertile Khat chewers (1.3, 1.11 nmol/ml respectively) compared with the fertile non-Khat chewers and fertile Khat chewers (1.0, 0.867 nmol/ml respectively, $P < 0.01$), while slightly lower TAC activity in infertile Khat and non-Khat chewers than in the fertile Khat and non-Khat chewers. MDA level was significantly lower while TAC level significantly higher in the overall Khat chewer group than in non-Khat chewer group. We also found Oxidative stress was

significantly associated with impaired sperm quality, P <0.05, and that Khat chewing decreased only sperm concentration.

Conclusions: We concluded, Oxidative stress is associated with male infertility and sperm quality gets badly affected by seminal oxidative stress. Khat does not increase seminal ROS level, however, it decreases sperm concentration. Seminal ROS and MDA levels can be utilized as a biomarker for male infertility.

KEYWORDS: Khat, Oxidative stress, Male infertility.

Background

Infertility is the inability of a couple to have a clinical conception or bring a pregnancy to term despite frequent unprotected sexual intercourse at least over the course of one year [1]. Male infertility is defined as the incapability of a man to produce spontaneous conception in a fertile woman. Infertility influences 13–20% of couples worldwide. As reported, male factor infertility impacts 5% of males in the reproductive age (puberty and 40 years) and between 25% -50% of couple infertility [2, 3, 4]. Infertility impacts both men and women; male infertility is most frequently due to various etiologies might be anatomical abnormalities, such as varicocele, tract obstruction, or neurological disorders of ejaculation that prevents the delivery of spermatozoa, or caused by a decline in semen parameters resulting of abnormal of spermatogenesis and dysfunction of spermatozoa which is a major contributor to poor fertility in most of the cases [4]. It has been estimated, the malefactor fertility arising due to abnormal semen parameters accounts for 40-50% of infertility [5, 6]. The mean incidence of unexplained male infertility is roughly 15% [2].

Although the development of scientific research and diagnostic approaches, the cause and pathogenesis of male infertility in some cases are still unexplained and compose the group of idiopathic infertility. At present, the etiology of suboptimal semen quality is still poorly understood, 30-40% of male infertility cases are

idiopathic and attributed to several intrinsic and extrinsic factors, such as smoking, alcohol consumption, chronic stress, obesity, urogenital trauma, and inflammation in the male reproductive system endocrine disorders, or genetic and epigenetic abnormalities as well as environmental factors such as exposure to certain chemicals, heavy metals, toxins, and heat, or ionizing radiation. It has been found, the after-effect of most of these causative agents is associated to oxidative stress they are generating that has a negative effect on sperm function [4, 5, 6]. Oxidative stress develops due to an imbalance between the production of reactive oxygen species (ROS) called free radicals and the reductive action of the antioxidant scavenging system responsible for the neutralization and scavenging of over-generated free radicals [2, 4]. Many studies reported that seminal oxidative stress is not only a major cause of poor sperm quality, even found also elevated in the normozoospermic idiopathic infertile men which cause spermatozoa DNA damage and also impacts the spermatozoa epigenome, resulting in infertility, recurrent miscarriage, and adverse pregnancy outcomes [3, 7, 8].

ROS are oxidizing free radicals and extremely reactive in an attempt to attain an electronically stable state by sequestering hydrogen from the adjacent carbon atoms, including superoxide anions (O_2•$^-$), hydroxyl (HO•), peroxyl (ROO•), and hydrogen peroxide (H_2O_2) radicals. Spermatozoa produce ROS in physiological amounts, which play an essential role in sperm capacitation, activation, acrosome reaction, and oocyte fusion [3, 2, 6]. However, oxidative stress, arises either due to overproduction of ROS as byproducts by energy-generating metabolic process, decreased total antioxidant activity, or both of them in the seminal plasma, results in male infertility by two ways; firstly, by oxidation of polyunsaturated fatty acids abundantly found in the plasma membrane of the spermatozoa, giving rise to a decline in sperm motility, viability, signal transduction, and in the ability of sperm to fuse with the membrane of the oocyte, and secondly, by damaging spermatozoa nuclear DNA and RNA, which finally produce defective spermatozoa that cannot contribute the paternal genome to the embryo [3, 6, 9]. Reported, oxidative stress may be implicated in 30–80% of infertile men cases [3]. Accordingly, a subtle

equilibrium between ROS needful for physiological activity and antioxidants to protect spermatozoa from oxidative damage is fundamental for fertility. Malondialdehyde (MDA) is a stabilized and final product of polyunsaturated fatty acids peroxidation and used as a biomarker for lipid peroxidation and membrane leakage caused by oxidants ROS as well as for the investigation of etiology of male infertility caused by oxidative stress [3, 9, 10].

Khat (Catha edulis) is a plant with evergreen leaves that grows in the southern Arabian Peninsula (in Yemen) and eastern Africa, where Khat leaves are typically chewed in social sessions lasting for several hours. Khat contains stimulating and euphoric agents, such as cathinone (a natural amphetamine), norephedrine, and norpseudoephedrine [11]. Although literature provided limited information about the influence of Khat chewing on oxidative stress generation and almost there were no research studies, up to our knowledge, that have evaluated its effect on the status of seminal oxidative stress and sperm quality among Khat chewers in Yemen. Studies showed, long term Khat chewing increased the activity of free radicals and lipid peroxidation in saliva of Khat chewers [11] compared with non-Khat chewers and in sera of Khat-consumers than non-consumers [12, 13]. A study revealed, Khat increases sperm count and motility in mice [14] while other showed it decrease sperm count and quality [15].

Despite the daily and widespread Khat consumption habit among the Yemeni people, approximately, there were no studies have been done to investigate the effect of Khat chewing on seminal ROS and TAC concentrations while only very few studies were inadequately investigated its effect on male fertility and were controversial. This study aimed at assessment of impact of Khat chewing and seminal ROS and TAC concentrations on spermatozoa quality and investigated whether Khat consumption has a role on seminal oxidative stress and male idiopathic infertility in Yemeni people. The specific objective of this study was to study the association between Khat chewing and levels of seminal ROS and TAC and sperm quality.

Methods

Participants and samples

We performed a case-control study to investigate the prospective effect of Kaht chewing on seminal ROS and total antioxidant capacity and their roles on male infertility. To investigate the effect of Khat chewing, participants were divided into two major groups; 50 infertile Khat and non-Khat chewers attended the andrology clinic at the Assisted Reproduction Centre in the Capital city Sana'a with a mean age of 32.40 ± 7.23 years as a case group, further subdivided into two subgroups: infertile Khat chewers group consisted of 25 patients and infertile non-Khat chewers group consisted of 25 patients. And 50 fertile Khat and non-Khat chewers with a mean age of 31.69±5.73 years, who had at least a child within the last year and their seminal analysis was normal according to 2010 World Health Organization (WHO) guidelines [16] as a control group, also subdivided into two subgroups: fertile Khat chewers group consisted of 25 men and fertile non-Khat chewers group consisted of 25 men. Informed consent and questionnaires were obtained from all participants enrolled in the study.

The study was approved by the Ethics Committee of the Faculty of Medicine and Health sciences - Sana'a University and conducted in accordance with the ethical standards laid down in the Helsinki Declarations. The study was conducted in a period of two years; the Khat chewers had chewed washed Khat daily for many years and for at least 4-hours a day. Further, no significant difference was noted in the age of infertile Khat chewers (31.80±7.36) and fertile Khat chewers (30.62±4.45), and also between the age of infertile non-Khat chewers (31.00±7.55) and fertile non-Khat chewers (32.88±4.45).

Male infertility cases recruited in the study suffered an infertility period for at least 12 months of unprotected regular sexual intercourse and had normal sperm concentration > or = 15 million/ml, normal semen volume > or = 1.5 ml/ejaculate,

negative sperm agglutination, healthy infertile males (free from any systemic diseases), fertile wives (examined for fertility by a gynecologist) in order to exclude the known male infertility causes and be able to attribute male infertility to the effect of oxidative stress and Khat chewing. All male infertility cases, who experienced semen volume < 1.5 ml and oligozoospermia (sperm concentration < 15 million/ml), or had smoking habits, alcohol use, orchitis, testicular trauma, sexually transmitted disease, varicocele, inguinal hernia operation as well as those have been using medications that affect male fertility potential within 3 months before enrollment in the study, were excluded from the study.

Sample Collection and Processing:

All participants were examined by an andrologist (Genital and Rectal digital examination, when needed). The semen samples were collected by masturbation after 3-7 days of sexual abstinence into a sterile, wide-mouthed container made of plastic. A portion of the samples was centrifuged and the supernatant (seminal plasma) was immediately separated and stored at -20°C for biochemical analysis. All semen samples were analyzed by two methods. Physical examination to measure semen viscosity and liquefaction period, microscopic examination using Makler chamber to evaluate sperm parameters, sperm concentration, and motility, using sperm smear for evaluation of sperm morphology, using eosin stain assess the sperm vitality according to the published WHO guidelines for references values for seminal fluid analysis parameters [16]. Biochemical assay with using a spectrophotometer at 534 nm to determine ROS level by measurement of seminal Malondialdehyde concentration using Thiobarbituric acid reagent (Sigma-Aldrich) (0.67 g of 2-thiobarbituric acid was dissolved in 100 ml of distilled water and into which 0.5 g NaOH and 100 ml glacial acetic acid was added) [17] and Total Antioxidant Capacity using a commercially available kit (Randox Laboratories Ltd, UK), the measurement procedure was conducted based on the manufacturer's instructions. Case samples were cultured to diagnose genital tract infection and prostatitis to exclude the infertility cases caused by bacterial infection.

Statistical analysis

Data were statistically analyzed using SPSS version 22. Quantitative variables were expressed as mean (±) standard error (SE). Descriptive Statistics was used to test for normality distribution of these variables. In normally distributed variables Independent-Samples t Test was used to compare the mean age of research groups and for two-group comparisons. 2 Sided P value ≤ 0.05 was considered to be statistically significant.

Results

Table (1) shows description and comparison of oxidative stress and semen parameters of Yemeni non Khat-chewing men aged 20-49 classified based on fertility.

Parameters	Fertile non-Khat chewers (control group) n=25	Infertile non-Khat chewers (case group) n=25
Volume (ml)	3.03±0.30	2.7±0.22
Sperm concentration (1×10^6)	81.46±9.70	71.28±12.10
Total Motility (%)	59.67±2.68	44.1±4.0 **
Progressive Motility (%)	46.50±2.8	30.12±3.59**
Non-Progressive Motility (%)	13.21±1.78	14.04±1.56
Normal Morphology (%)	15.17±1.39	2.44±0.32***
Vitality (%)	73.79±2.51	57.76±3.75**
MDA (nmol/ml)	1.00± 0.062	1.30± 0.033**
TAC (mmol/l)	1.73± 0.072	1.60± 0.090

* *P* <0.05**,** ** *P* <0.01, *** *P* <0.001

The results in table (1) shows, the mean of seminal MDA level in infertile non-Khat chewers was significantly higher when compared with the non-Khat chewers (1.30±0.033 and 1.00±0.037 nmol/ml, 95% CI -0.333 – -0.506, respectively, P <0.01). The mean level of seminal TAC in infertile non-Khat chewers was non-significantly lower than the fertile non-Khat chewers (1.6±0.090 and 1.73±0.054 mmol/l, 95% CI -0.164 – -0.304, respectively, P > 0.05). The mean levels of percent total motility (44.1±4.0 and 59.67±2.68, 95% CI 5.65 – 25.51, respectively, P <0.01), percent progressive motility (30.12±3.59 and 46.50±2.8, 95% CI 7.15 –

25.60, respectively, P <0.01), percent normal morphology (2.44±0.32 and 15.17±1.39, 95% CI 9.90 – 15.55, respectively, P <0.001), and vitality (57.76±3.75 and 73.79±2.51, 95% CI 6.86 – 25.19, respectively, P <0.01) were significantly lower, whereas semen sperm concentration were non-significantly lower in the infertile non-Khat chewers compared with the fertile non-Khat chewers. Semen volume and non-progressive motility volume did not make noticeable differences between the studied groups.

Table (2) shows description and comparison of oxidative stress and semen parameters of Yemeni Khat-chewing men aged 20-49 classified based on fertility.

Parameters	Fertile Khat chewers (control) n=25	Infertile Khat chewers (cases) n=25
Volume (ml)	2.09±0.23	2.7±0.28
Sperm concentration (1×10^6)	64.96±9.20	44.08±5.35*
Total Motility (%)	58.21±2.60	35.35±5.18 ***
Progressive Motility (%)	44.88±2.62	21.48±4.07***
Non-Progressive Motility (%)	13.88±0.91	13.87±1.49
Normal Morphology (%)	13.09±0.74	2.13±0.34***
Vitality (%)	71.71±2.61	53.13±4.40**
MDA (nmol/ml)	0.867± 0.032	1.11± 0.051**
TAC (mmol/l)	1.81± 0.067	1.74± 0.074

* *P* <0.05**,** ** *P* <0.01, *** *P* <0.001

The results in table (2) shows, the mean of seminal MDA level in infertile Khat chewers was significantly higher when compared with the fertile Khat chewers (1.11±0.051 and 0.867±0.032 nmol/ml, 95% CI -0.364 – -0.113, respectively, P <0.01). The mean level of seminal TAC in infertile Khat chewers was non-significantly lower than the fertile Khat chewers (1.74±0.074 and 1.81±0.067 mmol/l, 95% CI -0.139 – -0.226, respectively, P > 0.05). The mean levels of sperm concentration (44.08±5.35 and 64.96±9.20 $\times10^6$, 95% CI -0.725 – 42.64, respectively, P <0.05), percent total motility (35.35±5.18 and 58.21±2.60, 95% CI 11.32 – 34.39, respectively, P <0.001), percent progressive motility (21.48±4.07 and 44.88±2.62, 95% CI 13.71 – 33.08, respectively, P <0.001), percent normal

morphology (2.13±0.34 and 13.09±0.74, 95% CI 9.28 – 12.62, respectively, P <0.001), and vitality (53.13±4.40 and 71.71±2.61, 95% CI 8.37 – 28.78, respectively, P <0.01) were significantly lower, whereas both semen volume and non-progressive motility volume did not make noticeable differences between the studied groups.

Table (3) Description and comparison of oxidative stress and semen parameters of Yemeni men aged 20-49 classified based on Khat chewing.

Parameters	Khat chewers n=50	Non-Khat chewers n=50
Volume (ml)	2.40±0.18	2.9±0.18
Sperm concentration (1×10^6)	54.70±5.54	76.27±7.76*
Total Motility (%)	47.02±3.29	51.71±2.68
Progressive Motility (%)	33.43±2.93	38.14±2.55
Non-Progressive Motility (%)	13.87±0.86	13.63±1.10
Normal Morphology (%)	7.72±0.90	8.67±1.15
Vitality (%)	62.62±2.85	65.61±2.53
MDA (nmol/ml)	0.93± 0.032	1.16± 0.037**
TAC (mmol/l)	1.79± 0.050*	1.627± 0.062

* *P* <0.05**,** ** *P* <0.01,

The results in table (3) shows, the mean level of seminal MDA in Khat chewers was statistically significantly lower than that of non-Khat chewers (0.93±0.032 and 1.16±0.037 nmol/ml, 95% CI -0.234 – -0.029, respectively, P <0.01). The mean level of seminal TAC in the Khat chewers was significantly higher than that of the non-Khat chewers (1.79±0.050 and 1.627±0.062 mmol/l, 95% CI -0.065 – -0.239, respectively, P <0.05). Sperm concentration was significantly lower in the Khat chewers than that of the non-Khat chewers (54.70±5.54 and 76.27±7.76 $\times10^6$, 95% CI -40.64 – -2.48, respectively, P <0.05), whereas other parameters; percent total motility, percent progressive motility, percent normal morphology, and vitality and semen volume did not make noticeable differences between the studied groups.

Discussion

Oxidative stress has a negative impact on male fertility as they are believed to cause sperm dysfunction either by damaging sperm plasma membrane due to oxidation of polyunsaturated fatty or causing DNA fragmentation. This study focused on the association of oxidative stress to idiopathic male infertility through studying its effect semen parameters and spermatozoa quality. The study also was centered to evaluate the effect of Khat chewing on seminal ROS and total antioxidant capacity.

The level of seminal MDA concentration was significantly higher in patient groups, infertile non-Khat chewers and infertile Khat chewers (1.30, 1.11 nmol/ml respectively, $P < 0.01$) than that in the fertile non-Khat chewers and fertile Khat chewers (1.0, 0.867 nmol/ml), which denotes increased lipid peroxidation and elevated ROS level in seminal plasma in the patient groups in comparison with the control groups (fertile non-Khat chewers and fertile Khat chewers), *table (1 and 2)*. Based on the findings of the present study, we suggest, the oxidative stress in the patient groups is arising due to the intrinsic overproduction of ROS, which in turn cause negative impact on sperm concentration, spermatic function, sperm quality as well as causing sperm DNA damage and eventually leads to male infertility [7, 9, 8]. Our findings are consistent with the findings that found significantly higher seminal MDA concentration in infertile men than the fertile men [10, 16].

Increased ROS production could be attributed to defective oxidative phosphorylation resulted from dysfunctional mitochondria, ROS that is produced by cytoplasmic glucose-6-phosphate dehydrogenase (G-6-PDH) of spermatozoa, ROS that could be produced due to spermatic leucocytes producing up to 1000-fold more ROS, or low activity of TAC [7, 9].

The level of seminal TAC activity in patient groups, infertile non-Khat chewers and infertile Khat chewers (1.73, 1.81 mmol/l respectively, $P > 0.05$) was slightly lower than the fertile non-Khat chewers and fertile Khat chewers (1.60, 1.74 mmol/l

respectively P >0.05), *table (1 and 2)*. These findings infer, the seminal oxidative stress in infertile men subgroups was also due to the slight reduction in TAC concentration or TAC reduction might be attributed to the overconsumption of seminal TAC to detoxify the pathological ROS production in both patient groups. These findings were similar to some previous findings that found similar concentrations in both the fertile and infertile groups [18, 19].

Our findings in tables (1 and 2) showed, sperm count and sperm quality, including total motility, progressive motility, normal morphology, and vitality, were significantly lower in both patient groups, infertile non-Khat chewers and infertile Khat chewers, than those in the control groups, P <0.05. Hereby, we could infer, oxidative stress has been implicated in male infertility through reducing sperm function. These findings are consistent with the findings of some studies that found sperm quality was negatively impacted by increased ROS concentration in the seminal fluid [8, 10, 19, 20]. In some cases, men with normal semen parameters (normozoospermia) cannot fertilize their fertile women even when they have normal sperm function and this is due to DNA fragmentation caused by the elevated ROS concentration in semen [3, 7, 8].

The current study findings in the table (3) revealed the level of seminal MDA concentration was significantly lower in Khat chewers group than that in non-Khat chewers group (0.93 vs. 1.16 nmol/ml, P <0.01). Whereas, the level of seminal TAC was significantly higher in Khat chewers group than that in non-Khat chewers group (1.79 vs. 1.627 mmol/l, P <0.05). However, the study findings did not show any differences in sperm quality, involving total motility, progressive motility, normal morphology, and vitality between the Khat and non-Khat chewers, whereas sperm concentration was found significantly lower in Khat chewer group (54.70 vs. 76.27, P <0.05). Based on our study findings, Khat chewing does not increase seminal ROS level rather it increases TAC level to which the reduced ROS level in Khat chewer group may be attributed and this is because of the antioxidants substances existing Khat [21]. Our findings are not in agreement with the findings of some studies that

found long-term Khat consumption increased ROS level and decreased TAC level in human saliva and sera [11, 12, 13, 22]. This difference between our findings and of other studies may be attributed to whether the plant contains or is free of pesticides. Our samples have been collected from Khat chewers who consumed washed Khat. Sperm concentration decrease in Khat chewer group can be explained by that Khat was found in recent studies to reduce testosterone production and impairs spermatogenesis [15, 23], even though another study revealed the contrary, Khat doses stimulated sperm concentration and its motility [14].

Conclusions

Based on our study findings, male fertility and sperm quality are profoundly affected by oxidative stress due to the higher ROS production in semen. Although Khat chewing may reduce seminal oxidative stress, the results in our study did not find that it improves male fertility and this requires further studies to evaluate. Hereby, our results suggest that oxidative stress due to the increase of seminal ROS decrease of seminal MDA is a primary contributor to the etiology of male infertility. Therefore, they can be used as biomarkers for male infertility.

List of abbreviations

ROS: Reactive oxygen species

TAC: Total antioxidant capacity

MDA: Malondialdehyde

G-6-PDH: glucose-6-phosphate dehydrogenase

WHO: World Health Organization

CI: Confidence interval of the difference

P: P value

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee in the Faculty of Medicine and health sciences (No. 2008000420) – Sana'a University. The study was conducted in accordance with the ethical standards laid down in the Helsinki Declarations. Informed written consent and questionnaires were obtained from all participants enrolled in the study.

Consent for publication

All authors approve to publish this paper. Written consent to publish this information was obtained from study participants.

Availability of data and materials

The datasets analyzed in the current study are not publicly accessible; the study data were obtained from the original findings of the master's research of corresponding author.

Competing interests

The authors declare that there are no conflicts of interest

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Authors' contributions

A. A.M.A. has taken part in designing the study and conducted the experiment, including collection, microscopic examination and biochemical analysis of participant samples. As well as he has done the data analysis and written the article. R.M.J. has taken a part in designing the study, reviewed and proofread the article. F. K.A. has taken a part in designing the study, reviewed and proofread the article. M.M.A. has clinically examined all the participants, taken part in study design, reviewed and proofread the article. H.M.H.A., Z.A.A.A have taken a part in designing the study, reviewed and proofread the article. A.Z.A. has taken a part in sample collection and samples analysis. G.I.M. has taken a part in designing the study, reviewed and proofread the article. N.G.K. has taken a part in designing the study, reviewed and proofread the article. A.A.N. has taken a part in designing the

study, reviewed and proofread the article. Y.M.A. has taken a part in designing the study, reviewed and proofread the article. All authors have read and approved the manuscript.

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