The effects of Cannabis compounds (THC, CBD, and THC-COOH) on Sperm Motility in Male Participants: A Prospective Study

Ayham Ismaeil1, Peter Michael Jankowski1, Fatima Riffat Bibi2, Mohamad Eid Hammadeh1,3, Houda Amor1,3

Abstract

Objectives: The perception of Cannabis toxicity and safety in society, politics, and even scientific circles has changed dramatically in recent years. Consequently, a few questions have been raised regarding the effects of marijuana (Cannabis sativa) on the human body and psychic abilities in the short and long term. This study is undertaken in order to compare sperm cells quality before and after incubation with Cannabis (marijuana) extracts (THC-THC-COOH and CBD).

Materials and Methods: Semen samples were obtained from men (n=10) with age range between 20 to 35, regardless of their fertility status. Analyses of semen parameters (volume, sperm count, motility), were analyzed within 1 hour after collection, according to WHO criteria 2020. For accurate results, Computer Assisted Sperm Analysis (CASA) system was used to determine sperm parameters. Each sample was exposed to 1 mL; 10 µg/mL of THC, THC-COOH, and CBD dissolved in methanol. All samples were also exposed to 1 mL pure methanol as a control group to eliminate the potential effects of methanol on sperm cells. Several smears (10 µL) of each sample were taken in order to assess the functionality of sperm's chromatin integrity by employing a chromomycin (CMA) and DNA fragmentation (Acridine Orange) assays.

Results: After treatment sperm cells with 10µL THC, THC-COOH and CBD. Total sperm's motility was significantly reduced significantly when it was exposed to THC and CBD ($P \leq 0.001$ and $P \leq 0.003$ respectively). Progressive motility were decreased significantly ($P \leq 0.003$) after incubation with THC and CBD and respectively. However, slightly inhibition of total sperm's motility was observed after incubation with THC-COOH.

Conclusions: Spermatozoa exposure to THC, CBD and THC-COOH deteriorate significantly sperm motility and should be avoided by men in reproductive age and those who undergoing ART.

Keywords: Cannabis, Sperm, In-Vitro, THC, THC-COOH, CBD

Introduction

The past few years have witnessed a dramatic change regarding Cannabis toxicity and safety on the eyes of society, politics and even scientific field. Therefore, several critical questions about the short and long-term impact of marijuana (Cannabis sativa) on human body and psychic abilities have raised. Presently, Comprehensive discussions on global political and societal dimensions are taking place at both the state and federal levels regarding the potential legalization of Cannabis sativa or the employment of its derivatives for the alleviation of intractable medical conditions. (1) It is crucial for the scientific community to take on the responsibility of finding a suitable resolution to this ongoing debate. This can be seen clearly in Figure 1, which provides a visual representation of the issue at hand. A remarkable exponential surge in Cannabis research has occurred over the past two decades, reflecting the profound implications arising from the evolving discourse and legislative measures pertaining to the legalization of recreational and medicinal marijuana usage.

Cannabis (marijuana) is the most widely used illicit drug globally, and According to a survey conducted by the National Survey on Drug Use and Health (NSDUH), in 2019, approximately 19.4 million adults in the United States used marijuana in one month only. And according to a study published in the journal Addiction estimated that in 2015, global Cannabis consumption was between 128 and 238 million people. (3) Cannabis exhibits a multitude of psychological and physiological effects. As described in the 2008 World Health Organization report, these effects encompass alterations in perception and mood, impairments in psychomotor coordination and concentration, as well as physiological consequences such as elevated heart rate, reduced blood pressure, and regression of short-term and working memory (4). Long-term effects, however, have not been extensively substantiated by scientific research. Conversely, therapeutic applications of Cannabis have
displayed numerous well-documented beneficial outcomes. These encompass its utilization as a general analgesic, alleviation of nausea and vomiting, and enhancement of appetite in patients undergoing chemotherapy or those afflicted with acquired immunodeficiency syndrome (5).

The *Cannabis* plant comprises an array of chemical compounds, with the two most predominant: D9-tetrahydrocannabinol (THC), the mean psychoactive constituent in *Cannabis*; and cannabidiol (CBD), a cannabinoid that lacks the psychoactive implications of THC.

On the other hand, spermatogenesis represents an intricate and highly specialized procedure reliant upon the supportive microenvironment facilitated by testicular Sertoli and Leydig cells. The detection of cannabinoid receptors on these cellular components points toward a potential function for cannabinoids in maintaining the equilibrium of molecular signaling within this microenvironment. For instance, CB2 receptors are located on Sertoli cells, and modulating this receptor has demonstrated involvement in inducing apoptosis in these cells. Moreover, Leydig cells exhibit the presence of cannabinoid receptor CB1, which instigates a localized decrease in testosterone production, potentially influencing spermatogenesis (6).

*In vitro* examinations have investigated the consequences of THC on human sperm functionality. This specific cannabinoid exerts a dose-responsive adverse impact on progressive motility percentage, becoming more highlighted as semen quality diminishes overall. Furthermore, spontaneous acrosome reactions experience reduction, and THC demonstrates the capacity to impede the acrosome reaction even when artificially induced at both therapeutic and recreational plasma concentrations (7).

Contradictory findings pertaining to decreased testosterone levels, sperm production, sperm motility, and elevated sperm abnormalities (8) were later challenged by a more extensive and methodically rigorous study encompassing chronic-heavy users. This investigation revealed no discernable differences in plasma testosterone either at the beginning of the study or following three weeks of substantial daily *Cannabis* testosterone consumption (9). The purpose of the present prospective study was to determine the effect of THC, CBD substances along with THC-COOH, the central secondary metabolite of THC generated within the body upon consumption of *Cannabis*, on sperm motility, chromatin integrity and DNA fragmentation of living sperm cells.

**Material and Methods**

**Study Design**

Semen samples were gained from male participants (*n=10*), aged 20 to 35 years, irrespective of their fertility status. Medical histories and health conditions were not considered in this prospective study, as its primary focus was to evaluate the quality of spermatocytes pre- and post-incubation with a specified toxic substance. All volunteers were formally requested, via written correspondence, to grant permission for their inclusion in this research study.

**Sample Collection and Preparation**

Semen specimens were acquired via masturbation following a period of 2-7 days of sexual abstinence. These specimens were collected in sterile, wide, non-toxic receptacles and subsequently processed in the laboratory within 90 minutes post-ejaculation. Semen parameters, including volume, sperm count, and motility, were evaluated within two hours of collection in accordance with the 2020 WHO criteria. A Computer Assisted Sperm Analysis (CASA) system was employed to ensure accurate results for the assessment of sperm parameters.

Multiple smears (10 µL) of each specimen were obtained to evaluate sperm chromatin integrity through the utilization of chromomycin CMA, and acridine orange assays.

Following this, 2 mL of each sample was exposed to 1 mL of a soluble variant of THC, CBD and THC-COOH supplied in a 95% methanol solution by LoGiCal Germany, at a concentration of 1 mg/mL. All samples were also exposed to 1 mL 95% methanol as a control group to eliminate the potential effects of methanol on sperm cells. The target compounds were introduced directly into the semen, allowing sperm to swim under normal conditions and preserving essential nutrients.

Upon completion of a one-hour incubation period at room temperature, sperm analyses were carried out for control and treated semen samples. Both control and *Cannabis* -treated samples underwent identical experimental procedures.
Chromomycin CMA₃
Air-dried semen samples were fixed using a methanol-glacial acetic acid solution in a 3:1 ratio at 4°C for 60 minutes prior to being allowed to air dry at room temperature. Each slide was treated with 50 µL of CMA₃ staining solution, which contained 0.25 mg/mL CMA₃ in McIlvain's buffer at pH 7.0 and was supplemented with 10 mM MgCl₂. Subsequently, they were covered with coverslips and incubated in darkness for 30 minutes at room temperature. The slides were then rinsed with PBS buffer and mounted with a 1:1 (v/v) PBS/glycerol solution, before being stored at 4°C overnight. A total of 200 spermatozoa per slide were analyzed using a fluorescent microscope equipped with a 460-nm filter and 100x eyepiece magnification. The assessment of chromatin condensation states involved differentiating between spermatozoa exhibiting bright yellow stains (CMA₃ positive) and those displaying dull yellow stains (CMA₃ negative), as depicted in Figure 2 (10).

Acridine Orange
The integrity of chromatin in human spermatozoa was assessed through the application of Acridine Orange staining to predict DNA damage. This staining procedure was conducted in accordance with the Virant-Klun criteria utilizing the following methodology:
1. The slides were fixed for a two-hour duration in a freshly constituted Carnoy's solution (comprising Methanol and glacial acetic acid mixed in a 3:1 ratio) before being air-dried.
2. Acidic acridine orange solution was utilized for the staining of the slides.
3. The prepared slides were subsequently examined under a fluorescence microscope for analysis. In order to thoroughly evaluate the samples, a total of 200 spermatozoa were scrutinized from each slide, categorizing them based on their staining characteristics into either orange or yellow (acridine orange positive, indicative of denatured DNA) or green (acridine orange negative, representative of double-stranded and normal DNA) (11).

Statistical Analysis
Statistical analysis was performed using the SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA). In this study Post hoc comparisons were conducted using Mann-Whitney tests with a significance level set at P<0.05.

Results
The clinical data of semen and sperm analysis of all participants (n=10) as described in Table 1. The median of total sperm motility, progressive motility and immotile sperms were (68.7%, 24.4% and 31.3% respectively).

The percentage of normal morphology sperm were significantly correlated to sperm count and motility (P ≤ 0.001).

By introducing just 10 µL of THC, THC-COOH, and CBD to sperm cells, a significant reduction in their overall motility was observed. Both THC and CBD had a remarkable impact on sperm motility, with statistical significance values of P ≤ 0.001 and P ≤ 0.003 respectively. These findings highlight the potential detrimental effects of these substances on reproductive health. Conversely, only a slight inhibition of total sperm motility was noted following incubation with THC-COOH. Intriguingly, the quantity of immotile sperm significantly increased to the maximum when treated with THC and CBD (P ≤ 0.001 and CBD: P ≤ 0.003, respectively). Similar trends were detected in relation to progressive motility (THC: P ≤ 0.001 and CBD: P ≤ 0.004) (Figure 3).

No discernible alterations were identified in either morphology or chromatin's integrity of the sperm cells after a one-hour incubation period with the studied substances. This finding aligns with expectations, as modifications to chromatin and nuclear material typically transpire through gradual processes rather than occurring instantaneously.

Discussion
In the present study, a significant reduction in sperm motility following exposure to both THC and CBD was observed.

These findings are in accordance with a previous study conducted by Murphy et al who investigated the impact
of Cannabis compounds on male reproductive health and showed that an acute exposure to THC deteriorates sperm function and consequently fertilization potential (12). Furthermore, Ambrosi et al demonstrated that chronic exposure to low concentrations of THC imitated an endocannabinoid-related reduction in mouse sperm motility and viability (13).

Furthermore, the present research elucidated that exposure to CBD resulted in diminished sperm motility, even though to a lesser extent compared to the influence of THC.

Nevertheless, numerous studies have confirmed alterations in endocannabinoid signaling within male reproductive tissues, indicating that signaling modulation of male reproductive system by exogenous cannabinoids could have substantial consequences of sperm function (14).

This study examined the consequences of THC-COOH, THC, and CBD on male sperm (n=10) with participants aged twenty to thirty. Results revealed that both fractions exhibited diminished motile sperm quantities; however, THC and CBD inflicted more significant damage than sperm in the THC-COOH fraction.

The endocannabinoid system and its endogenous compounds, exhibits diverse physiological functions at both cellular and organ levels (15). A functional endocannabinoid system is present in various segments of the human reproductive tract, such as endometrium, ovary, epididymis, testis, sperm, and prostate. Identified endogenous agonists include anandamide (AEA), oleoylethanolamide, and palmitoylethanolamide, which are detected in reproductive secretions (16). Cannabinoid receptors have been demonstrated to influence menstrual cycles, implantation, embryonic development, lactation, and pregnancy maintenance in females (17). In contrast, information regarding the role of cannabinoid receptors in male fertility remains inadequate. However, recent studies provide indirect evidence for the existence of cannabinoid pathways in male sperm analogous to those found in the central nervous system by demonstrating the binding of labeled agonists (18-19).

In the literature scanty information in this regards could be found. The potential anti-motility effects of CBD provide an intriguing area for further investigation within this field, warranting additional research and potential interactions with THC and other cannabinoids.

This study provides compelling evidence and supports the findings on how THC negatively affects sperm motility.

**Limitations**

It is important to note that, there are Inevitably, certain limitations are associated with in vitro studies such as, a limited sample size, which can affect the statistical significance and reliability of the results. Not to forget, that in vitro studies often take place in a controlled and simplified environment, which may not fully represent the complex conditions of a living organism. This can limit the ability to extrapolate the results to real-life situations. Further research is needed to confirm these findings and determine the extent to which Cannabis use may impact male fertility. Nonetheless, these results highlight the potential risks associated with Cannabis use and underscore the need for caution when using this substance.

Additionally, it sheds light on a relatively unexplored area by examining the impact of CBD on sperm function. Further research such as understanding the mechanisms by which cannabinoids modulate sperm quality, investigating the epigenetic implications of cannabinoid exposure on male reproductive health, and assessing potential therapeutic strategies to counteract the detrimental effects of cannabinoids on male fertility, is required to unravel the complexities surrounding cannabinoid-driven disruptions in male reproductive health and developing an appropriate therapeutic intervention for afflicted individuals.

**Conclusions**

In the conducting study we have found that Cannabis compounds can have a significant impact on sperm motility in male participants. Our results suggest that the use of high-potency Cannabis can lead to a decrease in
sperm motility, which may have implications for male fertility. In conclusion, our study provides valuable insights into the effects of Cannabis on male fertility. We hope that our findings will contribute to a better understanding of the risks associated with Cannabis use and inform public health policies and guidelines.

Authors' Contribution
Data curation: Ayham Ismaeil, Fatima Rifat Bibi.
Formal analysis: Ayham Ismaeil.
Funding acquisition: Mohamad Eid Hammadeh.
Investigation: Ayham Ismaeil.
Methodology: Ayham Ismaeil, Houda Amor.
Project administration: Ayham Ismaeil, Mohamad Eid Hammadeh.
Resources: Ayham Ismaeil.
Software: Ayham Ismaeil, Peter Michael Jankowski.
Supervision: Houda Amor.
Validation: Mohamad Eid Hammadeh.
Visualization: Ayham Ismaeil.
Writing–original draft: Ayham Ismaeil.
Writing–review & editing: Houda Amor, Peter Michael Jankowski.

Conflict of Interests
The authors declare that they have no conflict of interest.

Ethical Issues
During the research process we protected the privacy of all participants; stringent measures have been put in place. Confidentiality of research data has been maintained at an adequate level, with secure storage and restricted access. Anonymity has been ensured for both individuals and organizations involved in the research, preventing any potential harm or discrimination. Informed consent was obtained from all the patients organizations involved in the research, preventing any potential harm or discrimination. Informed consent was obtained from all the patients involved in this study.

Financial Support
This study was supported by the Obstetrics and Gynecology Department, University of Saarland, Homburg/Saar, Germany.

References

© 2024 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.