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(RESEARCH ARTICLE)

Clastogenic effect by recreative exposure to marijuana in individuals from the Estado de Mexico: Pilot study

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Abstract

Cannabis sativa is the most trafficked illicit substance in the world, 147 million people consume it recreationally. This plant contains more than 100 phytocannabinoids, the most important being 1-delta-9- tetrahydrocannabinol (THC). The genotoxic effect of cannabinoids has been proven in vitro and in vivo models, where damage to nuclear DNA and chromosomal aberrations (CA) was observed. This shows the clastogenic potential of this drug. In this research, we study genotoxic damage in Mexican individuals recreationally exposed to marijuana. The study of chromosomal aberrations was performed in 48 hour lymphocyte cultures of 30 exposed and 30 no exposed individuals. 100 metaphases were analyzed per sample. The type of CA was identified and counted. The number of CAs identified in the exposed group was 344 with a median of 9.5; the no exposed group had 218 CAs, with a median of 6.5. The statistical analysis showed a significant difference between both groups, p=0.013. Chromatid and chromosomal breaks were the most frequent aberrations. Comparing the exposure time vs. frequency of CA in the exposed group, we did not find a significant difference between sub-groups from 1 to 5 and 6 to 11 years. Similarly, in the analysis of the last exposure date vs CA, no significant differences were observed between the subgroups <1 week and >1 week. Our results show an increase in the frequency of the number of CAs in the individuals recreationally exposed. These results show evidence of the genotoxic potential of marijuana.

Keywords: Marijuana; Chromosomal aberrations; Cannabinoids; Recreational use; Genotoxicity

1. Introduction

According to the World Health Organization (WHO), marijuana or *Cannabis sativa* is the most widely cultivated, trafficked and abused illicit substance in the world. Approximately 147 million people (2.5% of the world's population use it recreationall) y and 180.6 million report lifelong consumption [1, 2, 3]. However, there are no data on recreational use of this drug in Mexico.

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Marijuana contains more than 489 different chemical compounds and at least 100 phytocannabinoids [4]. Among those that stand out are cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN) and 1-delta-9-tetrahydrocannabinol (THC), which is the most abundant. Additionally, non-cannabinoid compounds are also found, such as alkaloids, terpenes, phenols, flavonoids and sugars. Most of these compounds are found in flowers, leaves and stems [4, 5, 6].

The metabolism of cannabinoids is carried out in the liver by the CYP2C9 enzyme subfamily of cytochrome P450, through hydroxylation and oxidation reactions. The elimination of these compounds is carried out by urine, feces, sweat, breast milk and saliva [7].

Marijuana is usually consumed in the form of cigarettes or in pipes. The absorption of phytocannabinoids is 40% by smoke inhalation, 95% of which binds to plasma proteins and can accumulate in the brain, kidney, stomach, lungs, liver, heart, spleen and adipose tissue. This causes a gradual release of these compounds into the blood for a long period of time [7].

The effect of recreational use of marijuana induces cannabinoids to activate mesocorticolimbic dopaminergic neurons, resulting in an increase in the level of dopamine to maintain pleasure or avoid pain [8].

Studies have reported non-neurological side effects from inhaled and prolonged use of marijuana, among which we find cardiovascular, respiratory and reproductive alterations [2, 9]. The genotoxic effect of *Cannabis sativa* has been proven in *in vitro* and *in vivo* models, where it was observed damage to the genetic material of the cell, such as chromosomal aberrations (deletions, translocations), errors in segregation, hypoploidy, inhibition of DNA and RNA synthesis and reduction of histone expression level. This shows the neoplastic and clastogenic potential of marijuana [10, 11].

However, the effect genotoxic or mutagenic effect of recreational consumption of *Cannabis sativa* is still controversial [10, 11, 12]. In addition to this, there are no reports of studies for its recreational use in Mexico. Therefore, the objective of this research was to study the genotoxic damage in lymphocytes of Mexican individuals recreationally exposed to marijuana.

2. Material and methods

2.1. Type of study

Comparative cross-sectional study.

2.2. Study groups

The study group consisted of 60 individuals, aged in a range of 18 to 30 years, apparently healthy, native of Toluca de Lerdo, Mexico. The exposed group consisted of 30 individuals who reported having used *Cannabis sativa* recreationally, in the form of cigarettes for a minimum period of 6 months. The no exposed group consisted of 30 individuals, who reported not having consumed it in any of its forms. A 5 mL sample of peripheral blood in a Vacutainer tube with heparin was taken from every participant, and was kept at room temperature until processing.

2.3. Ethical considerations

All participants voluntarily accepted. Informed consent was obtained from all individual participants included in the study. They answered the questionnaire on general data and information on marijuana use.

2.4. Lymphocyte culture

Cultures were performed in sterile 15 mL tubes, to which 5 mL of RPMI-1640 medium (Gibco), 0.25 mL of phytohemagglutinin (Sigma), 0.2 mL of penicillin-streptomycin 5000 U antibiotic (Microlab) and 12 drops of blood were added. The cultures were incubated at 37°C for 47 hours in a 5% CO_2 atmosphere. Then, 0.2 mL of colchicine 10 µg/mL (Sigma) was added and incubated for 1 hour at 37°C. Subsequently, it was centrifuged at 1,500 rpm for 10 minutes and the supernatant was decanted. 6 mL of hypotonic KCl solution (0.075 M) preheated to 37°C was added, the culture was resuspended and incubated at 37°C for 20 minutes. It was centrifuged at 1,500 rpm for 10 minutes and the supernatant was removed. The cell button was resuspended and 5 mL of Carnoy fixative (methanol: acetic acid 3:1) was added at 4°C under continuous stirring and incubated at 4°C for 30 minutes. It was centrifuged at 1,500 rpm for 10 minutes and the supernatant was discarded. Carnoy fixative washes were performed until a white cell button and transparent supernatant were obtained.

2.5. Determination of chromosomal aberrations

For each culture, drip slides were prepared on cold slides and stained with 10% Giemsa (Sigma) for 10 minutes. 100 metaphases were analyzed under a microscope, in which the number of structural and numerical aberrations was identified and quantified. The percentage of chromosomal aberrations in each individual was calculated.

2.6. Statistical analysis

Data analysis was performed showing a non-parametric distribution. The Mann-Whitney U test was applied to the number of AC between the groups. The statistical analysis was made in relation to exposure time and the last date of consumption in the group exposed to marijuana. The SigmaPlot software version 12.0 was used.

3. Results

3.1. Socio-demographic characteristics

The exposed group consisted of 26 men (86.6%) and 4 women (13.4%). The average age was 20.4 years. The average time of exposure to marijuana was 4.2 years. While the no exposed group was composed of 14 women (46.6%) and 16 men (53.4%). The average age was 21.7 years.

3.2. Chromosomal aberrations

The number of chromosomal aberrations identified in the exposed group was 344 with a median of 9.5; in the no exposed group, the number was 218 with a median of 6.5. The Mann-Whitney U analysis showed a significant difference between the study groups with a value of p=0.013. See Figure 1.

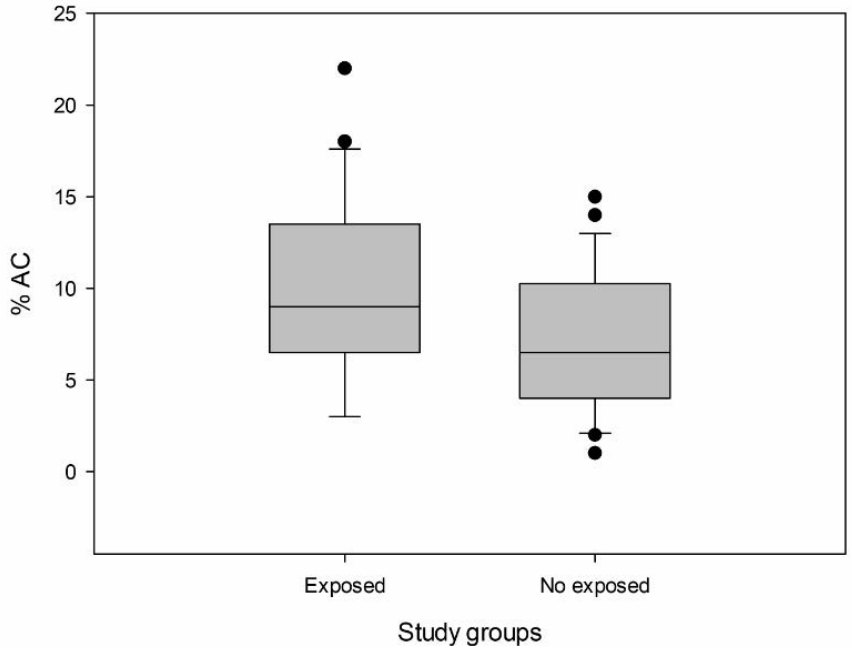

Figure 1 Results of chromosomal aberrations in the exposed group vs. not exposed.

Mann–Whitney U test, p=0.013

In the exposed group, the type of chromosomal aberrations identified were 214 (62%) chromatid fractures; 64 (19%) chromosomal fractures; 44 (12%) endoreduplication and 22 (7%) acentric fragments. In the no exposed group, the type of chromosomal aberrations were 140 (64%) chromatid fractures; 52 (24%) chromosomal fractures; 18 (8%) endoreduplication; 6 (3%) acentric fragments; 1 (0.5%) tetraradial and 1 (0.5%) tetraploid. The results of both study groups show that the highest frequency of chromosomal aberrations corresponds to chromatid and chromosomal aberrations. The increase in endoreduplication in the exposed group is noticeable, which corresponds to more than double those found in the no exposed group. The detail of the results is shown in Table 1.

Table 1 Chromosomal aberrations of the study groups.

Aberrations	Exposed	No Exposed
Chromatidic	214	140
Chromosomic	64	52
Endoreduplication	44	18
Acentric fragment	22	6
Tetraploid	0	1
Tetraradial	0	1
Total	344	218

3.3. Marijuana consumption

Based on the information obtained from the questionnaires in the exposed group about the use of marijuana, the following results were found. All participants consumed marijuana using cigarettes. The frequency of marijuana use was variable: 11 individuals (38%) consume it daily; 5 (17%) every third day; 4 (10%) once a week and 10 (35%) once a month. Spearman's correlation analysis between the number of CA regarding the frequency of marijuana use showed no significant association, r=0.321 with p=0.089.

On the other hand, regarding the amount of cigarettes consumed per occasion, it was found that: 22 (73%) individuals smoke 1 cigarette; 3 individuals (10%) 2 cigarettes; 3 individuals (10%) from 3 to 5 and 2 individuals (7%) more than 5. In relation to the number of AC and the amount of marijuana use, Spearman's correlation analysis showed no significant association, r=0.236 with p=0.208.

Two subgroups were formed according to the time of marijuana exposure: the first 1 to 5 years 21 individuals (70%) with a median chromosomal aberration of 8.0. The second subgroup considered from 6 to 11 years 9 individuals (30%) with a median aberration of 11.5. Statistical analysis between both subgroups showed no significant difference with Mann-Whitney U, p≥0.05.

Two subgroups were formed to analyze the frequency of CA vs. the last exposure date: the first group with less than a week 21 individuals (70%) whose median value of 10.0 and the second with more than a week 9 individuals (30 %) with a median CA of 11.4. Statistical analysis between both subgroups showed no significant difference with Shapiro–Wilk, p≥0.05.

4. Discussion

Usually, the cell has damage to the genetic material. This is mainly due to errors during DNA replication and/or induced by environmental factors. In both cases, most of these alterations are corrected, however, when corrections are not carried out rightly, they can give rise to several pathological alterations such as cancer or malformations [13, 14, 15].

Studies on the use of marijuana have focused mostly on neuropsychological effects, while there are few studies that have focused on non-neuropsychological effects, such as genotoxicity [2, 6].

Some authors report that there is no genotoxic damage induced by marijuana use, which is the case of Jorgensen *et al.*, (1991), who compared the frequency of SCE in lymphocytes of 22 tobacco smokers vs 22 tobacco and marijuana smokers and found no genotoxic damage [13]. On the other hand, García *et al.,* (1999), studied 30 students exposed to marijuana and 15 not exposed, and found no differences in the percentage of chromosomal aberrations between both groups [16].

The results of the present investigation show a greater number of chromosomal aberrations in the exposed group compared with the no exposed group. This is consistent with Hoyos *et al.,* (2001), who studied lymphocyte samples from 30 marijuana users and identified a greater number of chromosomal aberrations (chromatid and chromosome ruptures) when compared to a control group [12]. Similar results report Ammenheuser *et al.,* (1998), who conducted a study on 17 women who used marijuana and in the umbilical cord of their newborn children. They found a significant increase in genotoxic damage in mothers' lymphocytes and umbilical cords [10]. Recently, the study by Russo *et al.,*

(2019) shows that cannabidiol induces chain and apurinic site ruptures, DNA oxidation and micronucleus induction *in vivo* [17]. These investigations provide evidence of the cytotoxic, genotoxic and teratogenic potential of cannabinoids [18, 19].

Whereas cannabinoids enter the circulation and can be identified in whole blood with a concentration of up to 50% and an interaction with plasma proteins of 90%. This demonstrates the ability to distribute in different tissues, which together with their fat-soluble properties can be transported through the placenta and excreted in breast milk [20, 21].

It is known that cannabinoids are removed from the body within 30 days. The results according to time of use and last date of marijuana use, showed no significant difference between them. There is no data reporting on this in literature. An area that is desirable to investigate.

In the present study we found no significant difference between the years of exposure recreational to marijuana with the increase in chromosomal aberrations. It is possible that the size of the subgroups influences the results obtained, so it is advisable to increase the sample size. Considering that cannabinoids are fat soluble and stored in fatty tissue. It is desirable to quantify cannabinoids in plasma, serum, or urine to search for this association. There is no data on this objective in the literature.

Considering the limited information on the genotoxic effects of *Cannabis sativa* and the possible legal opening to consumption by the Mexican population, it is pertinent to continue with the study of its toxic effects, pondering genotoxicity, in order to provide factual information which will allow establishing the implications of recreational use on health.

5. Conclusion

The results of this research contribute to evidence that recreational consumption of marijuana induces chromosomal aberrations, which have been associated with the development of congenital malformations and a pre-neoplastic state. The results of this pilot study commit us to continue studying the genotoxic effect of recreational use of marijuana.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors state that they have no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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