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Original Article

Evaluation of socio-demographic characteristics of people was determined to use synthetic cannabinoids in Malatya and its surrounding cities / districts and LC-MS/MS analysis method

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Abstract

Aim: Synthetic cannabinoids (SC) were initially developed in 1960s to target the cannabinoid receptors in medical therapy. However, in recent years, the developed SC derivatives by processing the plant-based material have been sold on websites under the name of natural drugs.

The city of Malatya is located at the eastern part of Turkey and has an important location for the transfer and marketing of narcotic substances. In our study, presentation of the socio-demographic characteristics of the cases, the SC was determined in whose blood samples in the analyses, living in Malatya and the surrounding cities-districts were aimed.

Materials and Methods: In the scope of study, 275 cases whose blood samples was sent to the Chemistry Department in Forensic Medicine Institute at Malatya Group Presidency in 2016 and were detected as SC-positive by the chemical analysis were investigated.

Results: Out of 275 cases, 97.8% were male, 2.2% were female. Median age was 24, 66.5% (n=180) of them were primary school, 18% (n=51) were high school graduate and 0.7% (n=2) were university graduate. The educational status of 27 cases could not be obtained.

Conclusion: SC derivatives are marketed with slogan of "the natural ones are harmless", and their use among the young people is rapidly becoming widespread. In our study, it was determined that the SC users are in the young age group and have low educational status. In order to decrease the number of users, awareness meetings regarding the harmful effects of SC must be organized in the educational institutions.

Keywords: LC-MS/MS; MAM-2201; natural drugs; socio-demographic characteristics; synthetic cannabinoids; THC

INTRODUCTION

Synthetic cannabinoids (SC) were first developed in the research laboratories in 1960s with the aim of being used in the medical therapy through affecting cannabinoid receptors (1). John William Huffman and colleagues synthesized the compounds known as "JWH compounds" that have cannabinoid receptor activity (2). In 2004, SC were started to be sold on the websites as natural and legal drugs in attractive packages. The most commonly used and known names of them are "Spice" in Europe and "K2" in America (3-6). In case of Turkey, because of having the "Bonsai tree" icon on the packages and being absorbed into plant material, SC is named as "Bonsai". There are usually more than one SC derivatives responsible for the psychostimulant effect present in the Bonsai (3).

According to the data from the Substance Abuse and Mental Health Services Administration in United States of America (USA), in 2011, number of admissions to the emergency room due to the use of SC increased approximately 2.5 times compared to 2010 (4). According to the Center for Disease Prevention and Control, the number of admissions regarding the SCs in 2015 increased 229 % when compared to the admissions in 2014 (5). As reported by the European Drug Report 2016, SCs were

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stated to constitute the 60 % of new the psychoactive substances seized (7).

The most commonly used SCs used in Turkey had been JWH-018, JWH-073, HU-210 CP 47.497 until they were banned in Turkey on 13.02.2011. According to the "Turkish Drug Report" published in 2014 by *"Anti-Smuggling and Organized Crime Department", the number of SCs seized in 2013 increased 79.72 % compared to 2012 and 17 times compared to first time they were seized in 2011* (8).

The city of Malatya is located at the eastern part of Turkey and has an important transit and marketing location for the narcotic substances. In our study, we aimed to present the socio-demographic characteristics of the cases whose blood were analysed and determined as SC-positive living in Malatya and its surrounding cities and districts, to present the SC species used and to discuss the analysis method by using the liquid chromatography-tandem mass spectrometry (LC-MS/MS).

MATERIALS and METHODS

Chemicals

Standards for MAM-2201, MAM-2201 N-Pentanoic Acid (metabolite), MAM-2201-N-4-hydroxypentyl (metabolite), JWH-081, JWH-081 N-5-hydroxypentyl (metabolite), JWH-0814- hydroxypentyl (metabolite) and other 65 SC species were purchased from Lipomed (Arlesheim, Switzerland). D9-THC-d3 was acquired from Lipomed (Arlesheim, Switzerland). Methanol, ultra-distilled water, ethyl acetate and acetonitrile were from Merck (Darmstadt, Germany). Ammonium formate was purchased from Sigma - Aldrich (Taufkirchen, Germany). OASIS HLB cartridges were purchased from Waters (Waters, Milford Massachusetts, USA).

Preparation of Calibration Standards and Quality Control (QC) Samples from the Blood Samples

Blood samples without drugs were the base of the study. Adequate volumes of the solutions of N-Pentanoic Acid (metabolite), MAM-2201-N-4-hydroxypentyl (metabolite), JWH-081, JWH-081 N-5-hydroxypentyl (metabolite), JWH-0814- hydroxypentyl (metabolite) and other 65 SC species were added into the bloods in order to obtain a five-point calibration curve with the concentrations of 0.5, 1, 5, 10 and 25 ng/mL for each SC species. Calibration standards were prepared prior to each analytic measurement. Quality control (QC) samples indicating the lower limit of quantification and low QC (LQC), medium QC (MQC) and high QC (HQC) samples were prepared with the concentrations of 1, 2.5, 20 and 75 ng/mL substances in the blood by using different amounts of solutions of N-Pentanoic Acid (metabolite), MAM-2201-N-4-hydroxypentyl (metabolite), JWH-081, JWH-081 N-5-hydroxypentyl (metabolite), JWH-0814-hydroxypentyl (metabolite) and other 65 SC species. Controls for LQC, MQC and HQC were prepared in 1:4 human blood. The Upper Detection Limit (UDL) samples exceeding the high calibration point were prepared with the concentrations of 500 and 1000 ng/mL. All QC samples were prepared as a single group and stored at – 20 °C.

Solid Phase Extraction Method for the Blood Samples

Solid phase extraction was conducted by using OASIS HLB3 cc cartridges. Cartridges were conditioned with 2 mL of ethyl acetate, methanol and water, respectively. 1 mL of blood sample was diluted with 5 mL of water and centrifuged at 4400 rpm for 10 min and the transparent part on the top was transferred to the conditioned cartridges. Cartridges were then washed with 5% methanol (w/w), followed by drying under high pressure vacuum for at least 30 minutes. The elution step was completed by using 2 x 0.5 mL methanol and 2 x 0.5 mL ethyl acetate. The samples were then centrifuged at 14,000 rpm for 10 minutes and transferred to 0.2 mL LC-MS/MS inserts.

LC–MS/MS Analysis

Blood samples were prepared for the analysis on Shimadzu 8040 LC-MS/MS device. The column used was Ultra BiPh 3 µm x 50 mm x 3.0 mm (Restek). In the phase A and B of the mobile phase, ammonium formate/Water (10 mM) and 100% methanol was used, respectively.

Electrospray ionization was used for ionization. DL temperature, heat block temperature, drying gas flow (argon), total flow in pump flow through and pump B concentration were set to 250 °C, 400 °C, 15 L/min, 0.4 mL/min and 5 %, respectively. Column temperature was 40 °C. ESI mode of the MS device was run for positive and negative ion scanning. LC stop time was 16 minutes.

Device was used in multiple reaction monitoring (MRM) mode. Retention time and parent ions were determined by passing the standards through the system in the scanning mode. Then, Q1 was set to the parent ion and scanning was done in Q3. After the parent ion and product ions were detected, analyses were conducted according to the ratio of these ions. Then, the presence of another parent ions besides the parent ion were investigated by using the product ions. Presence of another parent ion was interpreted as the presence of a metabolite. Procedures applied for the parent ions were conducted for the new metabolites, as well. Analysis results were interpreted from the calibration curve produced after the determination of MRM method.

Synthetic cannabinoid species and names, ions and retention times of the compounds related with the THC are given in Table 1.

The analyses of the standards for HC, JWH-081, JWH-081 N-5-hydroxypentyl, JWH-0814-hydroxypentyl, MAM-2201, MAM-2201 N-Pentanoic Acid and MAM-2201 N-4-hydroxypentyl are presented in Figure 1.

Sample group

A group of 275 cases whose blood samples was sent to the Chemistry Department in Forensic Medicine Institute at Malatya Group Presidency by the legal authorities in 2016 and were detected as SC-positive by the chemical analysis were investigated in this study.

Table 1. Names, ions and the retention times of the detected compounds

Name of the compounds and their ions	Retention Time (RT) (Min)
THC : 345.2/299.2	6.73
JWH-081 : 372/185	
: 372/157	8.2
: 327/215	
JWH-081 N-5-hydroxypentyl: 388/185-157	7.0 (metabolite)
JWH-0814- hydroxypentyl: 358/171/143	7.79 (metabolite)
MAM-2201 :374/169-141	8.0
MAM-2201 N-Pentanoic Acid: 386/169/141	6.0 (metabolite)
MAM-2201 N-4-hydroxypentyl: 390/169-141	6.8 (metabolite)

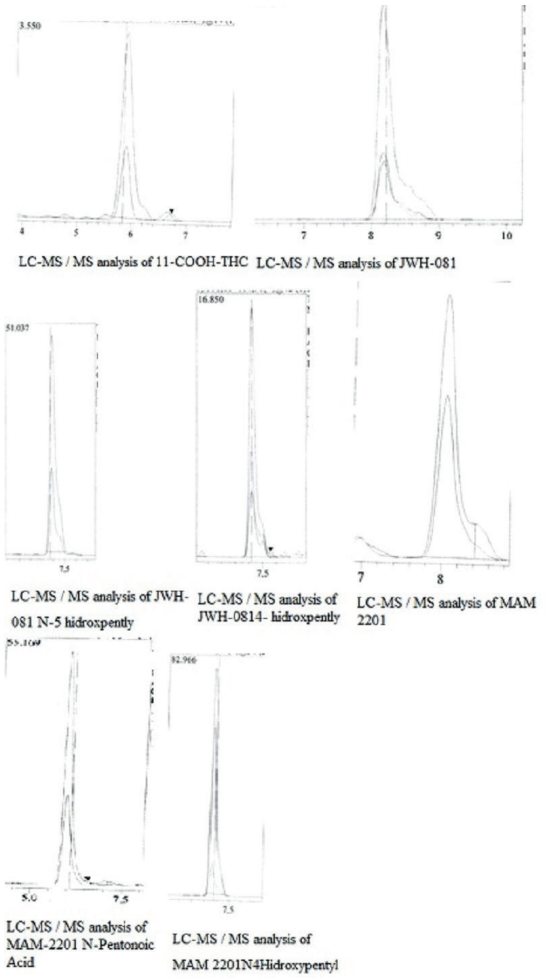

Figure 1. Results of the LC-MS/MS analyses of standard compounds

RESULTS

LC-MS/MS Analysis Findings

The distribution of the compounds detected in 275 cases are presented in Table 2.

Both MAM-220 and JWH 081 were detected in the blood samples of 189 cases (68.72 %). In 126 cases (45.81 %), both THC and SC were detected. No metabolites of JWH 081 were detected in the blood samples.

Table 2. Types of Detected Compounds, Number of Cases and Amount of Compounds

Detected Compound	Number of Cases Detected as Positive (%)	Amount of Compound (Detection range /mean)
THC	126 (45.8 %)	234-5 ng/mL / 59.54 ng/mL
MAM 2201	212 (%77.1 %)	296-1 ng/mL / 18.3 ng/mL
MAM-2201 N-Pentanoic Acid and MAM-2201 N-4-hydroxypentyl	262 (95.3 %)	904-2 ng/mL / 155.39 ng/mL
JWH 081	200 (72.7 %)	43-0.5 ng/mL / 6.36 ng/mL

Findings Regarding the Socio-demographic Characteristics of the SC Users

Out of 275 cases included in this study, 269 cases (97.8 %) were male and 6 cases (2.2 %) were female. The age of the users varied between 14 and 72 years. Median and mean age were 24 and 26.5 ± 9.2 years, respectively. The analyses of educational status revealed that 180 (65.5 %) of the cases were primary school graduates, 51 (18.5 %) of the cases were high school graduates and 2 (0.7 %) of the cases were university graduates. It was unable to reach the educational status of 27 cases (9.8 %). The legal authorities sending the cases were prosecutor's office (274 cases; 99.6 %) and the court (1 case; 0.4 %). 79 cases (28.7 %) were from Malatya and 196 cases (71.3 %) were from outside of Malatya. When the season of that the samples were sent in were considered, it was observed that 4 cases (1.5 %), 150 cases (54.5 %), 94 cases (34.2 %) and 27 cases (9.8 %) were sent in winter, spring, summer and autumn, respectively.

DISCUSSION

The Synthetic cannabinoids have similar effects with Δ9-tetrahydrocannabinol (THC) found in the cannabis plant. They bind to the cannabinoid receptors CB1 and CB2 and show fully antagonistic effects on them. The CB1, a member of the G-protein coupled receptor family found in central nervous system, is the responsible for the formation of the psychoactive effect. CB1 and CB2 receptors are found in many organs (9-13). CB2 is generally localized in the periphery and is not responsible for the psychoactive response (14). CB2 is found in the immune system and immune-mediated cells and is considered to be responsible for the anti-inflammatory effects (15). Due to the effect of SCs on CB1, the user feels relaxed and happy (4).

The most common way of use of the SCs in which more than one compound that have psychoactive effects

impregnated into the plant-based base material by spraying is through inhalation like cigarettes (1,6). Beside this, they can be used in the liquid form in the electronic cigarette cartridges, via evaporation, oral or rectal routes. The most important advantage of SCs is that they can be produced in vast amounts by using very small amounts of active compound (5,6,13).

When the SCs were launched, they were marketed to the users as being legal and not being detected in the routine drug tests. After the hazards of SC species were recognized and prohibitions were made, they were tried to keep in the legal limits and to be prevented from being detected in the chemical analyses by making changes in the molecular structures of the compounds. In the newly produced SCs, the parent molecule remains the same, but the side effects and the toxicity change due to the molecular changes made. While JWH-018 and AM-2201 were popular between 2010 and 2012, indole carboxylates and indazole carboxamides became popular in last years. The chemical structures of newly developed SCs revealed important differences in their cannabinoid receptor activity and metabolisms (16). JWH-081 is in the structure of cannabimimetic indole and its affinity to the central cannabinoid receptor is much higher (14). MAM-2201, a fluorinated SC species, is also among the newly produced SC species. The molecular formula of MAM-2201 is $C_{25}H_{24}FNO$ with the molecular weight of 374 g/mol (11, 17). MAM-2201 was reported in summer 2011 and quickly replaced JWH-018, JWH-122 and AM-2201 (17, 18). The most commonly SC species detected in our study was MAM-2201 and it was followed by JWH-081.

In Turkey, JWH-081, with decision of the Council of Ministers dated 07.01.2011 and Decision No. 2011/1310, and MAM-2201, with the decision of the Council of Ministers dated 05.05.2014 and Decision No. 2014/6330, were listed in prohibited substances list (19,20). Although MAM-2201 and JWH-081 were banned in 2011 and 2014, respectively, they were detected in the samples sent in 2016. This situation suggests the need of special devices to detect the substances and introduction of the standard substances to the device as they are not detected in the routine urine tests.

The studies about the prevalence of the SCs revealed that more than 11,000 people admitted to the emergency rooms in USA due to the SC use. Also, 75 % of the individuals admitted were the youngsters aged between 12 and 29. The number of admissions were increased to 28,531 in the following years. Approximately 80 % of the individuals admitted were males (21). In the 2017 European Drug Report, it was stated that the use of SC species especially threatens the 15-16 years old school aged kids. Ozturk et al. reported that 92 % of the samples sent by the legal authorities in Istanbul and detected as SC-positive were from males, while the 8 % of them were from females. The age range of the users was reported as 15-58 years (22). In our study, 98 % (269) of the cases were male and 2% (6) of the cases were female. This situation was thought to be due to the fact that the females do not participate in the social life as males and act timider in substance use in the Eastern Culture. The user age was between 14 and 72, and median age was 24. The age of starting the use of SC was found 14 years and it was found to be parallel with the present literature. Similar to the other studies conducted, it was found that the SC use was especially common among the young people, while various age groups use SCs. When the educational status of the users in our study was analysed, most of the cases (180; 65.5%) were primary school graduates and 51 cases (18.5) were graduated from high school. Together with that the median age of the users were 24 years, it was observed that the educational status of most of the users were lower. Having higher educational status were found protective as individuals with higher educational status have adequate knowledge about drug use and its damages.

When the locations that the SC users were living were analysed, it was observed that 78 of the cases (28.4 %) were living in the city center, while 197 of them (71.6 %) were living in the country towns. SC use was found to be intense in the districts where the statutory audit is not strict.

There were 126 cases that both THC and SC were detected in. It was thought that these cases were using SCs together with cannabis to try SC and during their transitional period. There were both MAM-2201 and JWH-081 in the blood samples of 189 cases. This situation was thought to be because the dealers were mixing different SC species in order to increase the psychostimulant effects.

The order of seasons in which the samples were sent from the highest number to lowest was spring with 150 cases (54.5 %), summer with 94 cases (34.2 %), autumn with 27 cases (9.8 %) and winter with 4 cases (1.5 %). The reason of the increased number of cases during the spring and summer was thought to be due to the increased social interactions and communications between the users especially in these two seasons.

There are different approaches regarding the ban of SC species. The first one of these approaches is the ban of the active compound itself, while the other one is the addition of the new derivatives of the compound to the list. While the chemical formula of the compounds was in the list of banned drugs during the first years, because the new species have been launched every year, compounds with similar molecular structure have also been started to be banned in USA. In Turkey, new SC species have been added to the list of drugs banned by the decision of the Council of Ministers. The current situation in Turkey legalizes the use of drugs until the decision to ban is taken. Drug use was legal until a decision to ban in Turkey but facilitated the inclusion of new psychoactive substances on the drug in Turkey and response time has been significantly shortened by the "generic classification" that entered the legal newspaper and came into force with the number 29259 dated February 6, 2015.

CONCLUSION

The Synthetic cannabinoid species have been marketed with the slogan of "Natural ones are harmless" and use of it is rapidly becoming widespread among young people. In our study, the users of SC species were in the young age group with lower educational status. In order to decrease and prevent the use of SC, social policies including the serious educational activities regarding the harmful effects of SCs should be developed besides the criminal methods available.

Conflict of interest : The authors declare that they have no competing interest.

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