New Psychoactive Substance 5-MeO-MiPT In vivo Acute Toxicity and Hystotoxicological Study

Yusuf Ali Altuncı1, Melike Aydoğdu2, Eda Açığöz3, Ümmü Güven4, Fahriye Duzağa5, Aslı Atasoy5, Nebile Dağlıoğlu5, Serap Annette Akgür5

1Department of Emergency, Ege University School of Medicine, İzmir, Turkey
2Department of Addiction Toxicology, Ege University Institute on Drug Abuse, Toxicology and Pharmaceutical Science, İzmir, Turkey
3Department of Histology and Embryology, Yüzüncü Yıl University School of Medicine, Van, Turkey
4Department of Stem Cell, Ege University Health Science Institute, İzmir, Turkey
5Department of Forensic Science, Çukurova University, Institute on Addiction and Forensic Science, Adana, Turkey

Address for Correspondence: Yusuf Ali Altuncı, Department of Emergency, Ege University School of Medicine, İzmir, Turkey
+90 232 390 23 25
yusuf.ali.altunci@ege.edu.tr

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Background: The hallucinogenic tryptamine analog 5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT) causes social problems worldwide. There are several studies on the metabolism; however, not more studies were found in the literature on acute toxicity.

Aims: We report the acute toxicity of 5-MeO-MiPT in mice, followed by quantitative toxicological analysis of blood and organs, hystotoxicological and immunohistochemical analysis of tissues and cells.

Study design:
In vivo experiments were performed using CD1 adult female mice (n=26). Animals were caged in 4 groups randomly.

Method: First group was a control (n=3). Second group was vehicle control (n=3) and injected 150 µL of blank solution (50% dimethyl sulfoxide in saline /0.9% of NaCl). While for acute toxicity experiments, 5-MeO-MiPT was added to a blank solution in order to obtain a dose of 0.27 mg/kg in 150 µL injection (n=10) and the last group were injected 2.7 mg/kg 5-MeO-MiPT in a 150 µL injection (n=10). Quantitative toxicological analysis, hystotoxicological and immunohistochemical analysis were performed.

Results: In the toxicological analysis, 5-MeO-MiPT was found negative in biological samples which were control, vehicle control, and 0.27 mg/kg dose mice groups. 5-MeO-MiPT was found 2.7-13.4 ng/ml in blood, 11-29 ng/g in kidney, 15.2-108.3 ng/g in liver, and 1.5-40.6 ng/g in the brain in 2.7 mg/kg injected group.

In a low dose of the 5-MeO-MiPT liver section, compared with normal tissues, the difference in staining was statistically significant (p<0.0001). In high-dose of 5-MeO-MiPT, H-score showed that the increase in the number of Caspase-3 positive cells was significant compared to the control (p<0.05). In high-dose of 5-MeO-
MiPT, intense Caspase-3 immunoreactivity was observed and the increase in the number of Caspase-3 positive cells compared to the control was statistically significant (p<0.05). In brain section, the statistics of the results obtained from the H-score showed that the increase in the number of Caspase-3 positive cells was significant compared to the control (p=0.0183).

In vehicle control liver section, there were few Caspase-8 positive cells characterized by a light brown appearance (p=0.0117). In the high-dose 5-MeO-MiPT group, the numbers of positive cells at low and high doses of 5-MeO-MiPT group were statistically significant compared to the control (p<0.05). In the high-dose 5-MeO-MiPT group, Caspase-8 immunoreactivity was detected in the glomerular structures. Compared to control, the increase in Caspase-8 immunoreactivity was found to be statistically significant (p<0.05).

**Conclusion:** Low-dose 5-MeO-MiPT did not cause any serious histopathological effects on the liver, kidney, and brain. High doses induce apoptotic cell death through caspase activity.

**Keywords:** Forensic toxicology, New Psychoactive Substance (NPS), Tryptamine, 5-MeO-MiPT, Moxy

**Introduction**

New psychoactive substances (NPS) are new, abusive narcotic or psychotropic substances that can threaten public health and safety (1–3). As the chemical structure of NPS differs from the stimulating substances, the legislation for these substances is constantly updated (4,5). Products often called “bath salts”, “not for human consumption”, “legal”, “deodorizing” and “plant food”, such as providing attractive contents of vague descriptions are sold in colorful packaging. Also, “health certificate” is marketed on websites showing like safe and legal (2,4, 6–8). Between 2008 and 2018, a total of 434 different NPS were reported by East and South East Asia countries. New psychoactive substances are frequently synthesized from derivatives of many synthetic tryptamine and then used for recreational purposes(9-11). As a result of the constant changes in the illicit drug market and the difficulty of detecting these substances through routine analytical methods, the estimated number is likely to be higher. For the last years, an amount of 40 N, N-dialkylated tryptamine derivatives were identified analytically(12).

5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT) is an analog of the popular drug 5-Methoxy-N,N-diisopropyltryptamine (5-MeO-DiPT, Foxy) and it is named “Moxy” (11). “Repke et al and Shulgin and Shulgin” made the first reporting of 5-MeO-MiPT synthesis and psychoactive effects .. Doses of 4–6 mg was reported to be used for oral consumption and for administration via smoking, higher doses of 12–20 mg were used. It has been found that, a 5-methoxy group’s attachment to the potency tryptamine core has increased and decreased the effects on perceptual changes in appearance. It has also been reported that N-substitutions have an effect on tryptamines' in vivo potency (13).

5-MeO-MiPT may be administered by inhalation or orally. The dose for oral administration is 4 to 6 mg and the period of effect is 4 to 6 hours if inhaled, the dose is 12-20 mg. The psychoactive effects begin 15 to 20 minutes after oral ingestion and reach a peak value between about 45-60 minutes. After about 10 hours later, the effect starts to decrease and recovery is recorded. If smoked, the duration of effects is 2 to 5 hours at the time of onset, and after around 2-4 hours, symptoms disappear. Effects such as euphoria, visual distortions, increased sense of touch, relaxation, and difficulty in sleeping have been reported by users (10,11,14).

5-MeO-MiPT, like many other tryptamine derivatives, is an agonist of the 5-HT2A receptor (15). It inhibits 5-HT and norepinephrine re-uptake, with less dopamine re-uptake (3,16). 5-MeO-MiPT has a relatively rare and short usage history (14). However, different reports published recently mention the increasing popularity of this substance (16,7,17).

To date, publications on 5-MeO-MiPT consist of receptor interaction profiles (15), receptor functional data (18), case reports and determination of NPS (14,19), and studies of metabolite determination by advanced analytical methods (12,20).

In this paper, we report the acute toxicity of 5-MeO-MiPT in mice, therefore, mice were injected intraperitoneally 5-MeO-MiPT, followed by quantitative toxicological analysis of blood and organs, histotoxicological and immunohistochemical analysis of tissues and cells.

**MATERIAL AND METHODS**

**Reagents and chemicals**

The reference standard of 5-MeO-MiPT was bought from Lipomed (Cambridge, US). Internal standard diazepam-d5 was purchased from 10 µg/ml in methanol; Restek (Bellefonte, PA). HPLC-grade methanol (MeOH) and water, sodium chloride, acetone, (NaCl), dimethyl sulfoxide (DMSO), ammonium hydroxide (NaOH), ethyl acetate, ultrapure methanol were bought from Merck Darmstadt, Germany). Ammonium formate and phosphate buffer 0.1 M, pH 4.4) was purchased from EMD Chemicals (Gibbstown NJ, USA).

Formaldehyde, ethanol, hydrogen peroxide (H₂O₂) were bought from Merck (Darmstadt, Germany). Phosphate-buffered saline ((PBS) pH:7.4) was purchased from Thermo Fisher Scientific (USA). Hemanotylin&KoeSine (H&E) was purchased from Sigma-Aldrich (Darmstadt, Germany). Tryptsinized (2% trypsin in 50 mM Tris
PBS for 15 min. Sections were trypsinized (2% trypsin in 50 mM Tris buffer) for 20 min 37°C. Then circles
After sectioning, samples were deparaffinized, immersed series of ethanol and washed first ultrapure water then
Immunohistochemical Analysis
samples were visualized by using an Olympus BX-51 microscope (Olympus, Japan).
Histopathological Analysis
processing were applied. Then 5 μm thick sections were cut from every sample and blemished with H&E. All
After removing the organs, organ parts was put into 4% formaldehyde in 0.1 M PBS and histological tissue
collect 60 min after vehicle injections (20). Blood samples collected from mice heart and abdominal cavity.
Statistical Analysis
samples were evaluated by three different times independently and blinded to the final score.
activity was applied on tissues 3% H2O2 for 15 min. Sections were incubated with Caspase-3 and Caspase-8 in a
humidity chamber at 40°C for overnight. Then sections were incubated with biotinylated secondary antibody for
1 h and streptavidin bound horseradish peroxidase for 1 h.  Sections were incubated diaminobenzidine (DAB).
Semi-quantitative Analysis of The Immunoreactivity Caspase-3 and Caspase-8
Semi-quantitative assessment of Caspase-3 and Caspase-8 immunoreactivity was determined using H-score
which involves a semiquantitative assessment of the percentage of positive cells and the intensity of staining
(graded as: 0, non-staining; 1, weak; 2, median; 3, strong) both.  H-scores were obtained by the formula: 1 × (%
of 1 + cells) + 2 × (% of 2 + cells) + 3 × (% of 3 + cells). The range of possible scores was from 0 to 300. A total
of 10 randomly selected caspase-3/caspase-8 stained images were counted. The number of positive cells was
determined by scoring at least 100 cells per 10 view-fields of tissue sections while magnified at ×20. The
samples were evaluated by three different times independently and blinded to the final score.
Ethics Approval
In vivo animal studies were performed by using 26 adult female CD1 mice, 8-10 weeks old, weighing 20-30 g
(Kobay Experimental Animals Laboratory A.S., Ankara, Turkey). The study protocol was approved by the ethical
committee of for Animal Experiments of the XXX University (Ref. 2017-115). All mice were
maintained controlled in a 12 h light / 12 h dark cycle, 24°C, 60% humidity conditions, and all fed ad libitum.
Animals were caged in 4 independent groups by completely randomized design. The first group was a control
(n=3), and nothing was injected. The second group was vehicle control (n=3) and injected 150 μL of blank
solution 50% dimethyl sulfoxide in saline (0.9% of NaCl). While for acute toxicity experiments, in order to get a
dose of 0.27 mg/kg in a 150 μL injection (n=10) (20),5-MeO-MiPT was added to a blank solution and the first
group was injected 2.7 mg/kg 5-MeO-MiPT in a 150 μL injection (n=10). All injections were applied intraperitoneal (ip).
The 0.27 mg/kg dose was estimated according to a rough quantification of 5-MeO-MiPT in pill samples in a study (20) and the same dose was administered in this study. Also, the 2.7 mg/kg dose was estimated and administered based on pharmacological studies. After 1 hour, mice were quickly sacrificed by cervical
dislocation. For the control group (three animals), and the vehicle control (three animals) blood samples were
collected 60 min after vehicle injections (20). Blood samples collected from mice heart and abdominal cavity.
Brains, kidneys, and livers were removed and stored at -80°C until toxicological, histopathological and
immunohistochemical analysis was performed. ARRIVE (Animal Research: Reporting of in vivo Experiments)
guideline was used in the reporting of animal experiments. (21)
Sample treatment of histopathological and immunohistochemical analysis
Histopathological Analysis
After removing the organs, organ parts was put into 4% formaldehyde in 0.1 M PBS and histological tissue
processing were applied. Then 5 μm thick sections were cut from every sample and blemished with H&E. All
samples were visualized by using an Olympus BX-51 microscope (Olympus, Japan).
Immunohistochemical Analysis
After sectioning, samples were deparaffinized, immersed series of ethanol and washed first ultrapure water then
PBS for 15 min. Sections were trypsinized (2% trypsin in 50 mM Tris buffer) for 20 min 37°C. Then circles
were drawn around tissue on slides with hydrophobic barrier pen and inhibition of endogenous peroxidase
activity was applied on tissues 3% H2O2 for 15 min. Sections were incubated with Caspase-3 and Caspase-8 in a
humidity chamber at 40°C for overnight. Then sections were incubated with biotinylated secondary antibody for
1 h and streptavidin bound horseradish peroxidase for 1 h. Sections were incubated dianminobenzidine (DAB).
After counterstaining with Mayer′s hematoxylin sections were washed and visualized by using an Olympus BX-
51 microscope (Olympus, Japan).
Semi-quantitative Analysis of The Immunoreactivity Caspase-3 and Caspase-8
Semi-quantitative assessment of Caspase-3 and Caspase-8 immunoreactivity was determined using H-score
which involves a semiquantitative assessment of the percentage of positive cells and the intensity of staining
(graded as: 0, non-staining; 1, weak; 2, median; 3, strong) both.  H-scores were obtained by the formula: 1 × (%
of 1 + cells) + 2 × (% of 2 + cells) + 3 × (% of 3 + cells). The range of possible scores was from 0 to 300. A total
of 10 randomly selected caspase-3/caspase-8 stained images were counted. The number of positive cells was
determined by scoring at least 100 cells per 10 view-fields of tissue sections while magnified at ×20. The
samples were evaluated by three different times independently and blinded to the final score.
Statistical Analysis
Caspase-3 and Caspase-8 immunoreactivity was calculated by statistical analysis. The Shapiro-Wilk was used to
test the normality of the distribution of the data. All groups passed the normality test (p>0.05), details were seen
at Supplementary File. Quantitative data are presented as the means ± standard deviation (SD) of three
independent experiments. Caspase-3 and Caspase-8 immunoreactivity compared by 2-way ANOVA due to more
than one factor (between the subject: two different doses and within the subject: tissue sections) followed by a
Tukey post-hoc test using GraphPad Prism 8 (Graphpad Software, Inc). Differences were accepted significant if
the value of p<0.05.
Based on data from pilot study, an analysis withwas performed for sample size estimation, and published study
Fabregat-Safont et al.by using PS: Power and sample size program. The Type I error probability for a two sided
test (α) 0.05; a difference in population means (δ) 0.3; standard deviation (σ) 0.03; ratio of control to
experimental patients (m) 0.25 were used and power was calculated 0.999. Thus, our sample size of N=26 (3
control, 3 vehicle control, 10 low dose, 10 high dose) will exceed the needed amount  for the main goal of this
study.
Sample treatment of toxicological analysis

Extraction of Blood Samples
Totally 200 μL of the blood sample was obtained. Blood samples were added 10 μL internal standard (diazepam-d5) and 1 ml ultrapure water. Then, samples were centrifuged for 10 min at 3500 rpm and passed through an Oasis® HLB (3cc, 60 mg) solid-phase extraction (SPE) cartridge. Blood samples were applied to a cartridge that had been conditioned with 2 mL methanol and 2 mL ultrapure water all at flow rate 1mL/min. The samples were passed through the cartridges at a flow rate of 0.5 ml/min, which is low and then washed in ultrapure water by using 2 mL 5% methanol and dried under vacuum at approximately 125 mHg for 5 min. The analytes were eluted with 2 mL acetonitrile: methanol mixture (70:30 v/v) and 2 mL 2% NH₄OH in ethyl acetate 98:2 v/v). The collected eluate was concentrated to dehydration with a mild flow (125 mHg) of nitrogen gas for 5 min. The residues were formed again up to in 200 μL methanol and 20 μL injected onto the liquid chromatography with tandem mass spectrometry (LC-MS/MS) system.

2.4.2. Extraction of Tissue Samples
Each tissue sample was thawed at room temperature and aliquoted about approximately 2-3 g. 100 mg of sample was homogenized in phosphate buffer (0.1 M, pH 4.4) (IKA Ultra Turrax Tube Driver). 10 μL of internal standard (diazepam-d5) was spiked to 1 mL of the homogenate taken from each homogenized tissue sample. Homogenates were dissolved again in 4 mL phosphate buffer. Later, the samples were centrifuged at 3500 rpm for 10 minutes and the supernatants were taken. Drugs were extracted from tissue samples by using the same extraction procedure with blood samples. 20 μL of each sample was injected into the LC-MS/MS system.

Instrumentation
All experiments were performed on Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) for chromatographic analysis. LC-MS/MS analysis was performed on a Shimadzu CBM-20A Ultra Flow Liquid Chromatography UFLC with a Shimadzu SIL-20A/HT autosampler system and Shimadzu Q-TOP LC-MS/MS systems (ESI-quadrupole mass spectrometer). The samples were separated by using a pentafluorophenylpropyl (PFPP) column Allure 50x2.150mm i.d., 5 μm, (Restek, Bellefonte, PA, USA), and maintained at ambient temperature. The mobile phase consisted of ultrapure water with 10 mM ammonium formate (solution A) and methanol (solution B). The gradient was started for the positive mode with 10% B, and held for 10 min at flow rate 0.5 mL/min, then the flow rate increased to 1 mL/min with 90% B and kept at these conditions for 5 min, after 15 min the flow rate returned to 0.5 mL/min with 10% B. HPLC eluent was diverted to waste for the first 1.5 min. The total run time per sample was 18 min. LC-MS/MS parameters established for the MRM acquisition mode is given (Table 1 and Fig. 1).

Working solutions were prepared by diluting the reference standards in different ratios with methanol that was used for the preparation of the calibration points. Spiking 190 μL blank blood with 10 μL of working solution, calibration samples with concentrations of 1, 2.5, 5, 10, 25, 50 ng/mL were obtained. For tissue samples, spiking 190 μL blank homogenizing with 10 μL of working solution, calibration samples with concentrations of 1, 2.5, 5, 25, 50 ng/g were obtained.

RESULTS
Histological Results
Histological examination of the mice in control and vehicle controls showed the normal architecture of hepatic lobules consisting of hepatocords radiating from a central vein towards the lobular periphery, portal vein and sinusoids (Fig. 2A and 2B). After administration of 5-MeO-MiPT, it was determined that the normal histological structure of the liver was preserved (Fig. 2C and 2D). Only mild non-specific immune cell inflammation was observed, especially in the portal track areas and central vein in low and high doses of 5-MeO-MiPT (Fig. 2C and 2D). At larger microscopic magnification, lymphocytes between red blood cells appear as dark-stained objects. The dark-stained (basophilic) staining pattern corresponds to the nuclei of the lymphocytes (Fig. 2C and 2D). In addition, a mild degree of degeneration and cytoplasm swelling of the hepatocytes characterized by a more pale pink color were seen in a high dose of 5-MeO-MiPT (2.7 mg/kg) (Fig. 2D).

Kidney sections of all groups’ mice showed normal histological structures of the glomeruli and renal tubules (proximal and distal) in the cortical and medullary portion (Fig. 3). The glomerulus is the conspicuous a tiny ball-shaped structure. Deformations of glomerulus structures of the kidney were determined in a high dose of 5-MeO-MiPT (2.7 mg/kg) (Fig. 3D). At the high dose of 5-MeO-MiPT, although not very obvious, glomeruli shrinkage and dilatation of Bowman’s spaces were observed.

Light microscopic examination of H&E dyed sections of the brain tissues of different groups of animals is depicted in Fig. 4. Brain tissue sections of mice showed normal neurons with dense basophilic cytoplasm. After the administration of 5-MeO-MiPT, no histological alterations were detected.

Immunohistochemical Results
The immunohistochemical assay was used to determine the expression patterns of Caspase-3 and Caspase-8 in kidney, liver, and brain tissues. A 2-way ANOVA was used to compare staining intensities of Caspase-3 and Caspase-8, and a Tukey’s post-hoc test to determine important differences between individual intensities. Cascase-3 and Caspase-8 immunoreactivity were evaluated considering brown (DAP) and blue (hematoxylin)
staining areas. The brown DAP staining pattern showed positive caspase-3 and caspase-8 immunoreactivity. In liver, kidney and brain sections, there was no expression of Caspase-3 in the observed normal tissues as shown in Figure 5A, 6A, and 7A. In vehicle control liver section, Caspase-3 staining appeared similar to that of normal tissues (Fig. 5B), no statistically significant difference was shown in the results obtained as a result of the semi-quantitative evaluation (Fig. 11) (p>0.05). In a low dose of the 5-MeO-MiPT liver section, it was found that the number of Caspase-3 positive cells, whose cytoplasm was stained in brown, as shown in Figure 5C, was quite low. Compared with normal tissues, the difference in staining was statistically significant (Fig. 11). When being exposed to high-dose of 5-MeO-MiPT, significant Caspase-3 immunoreactivity was observed in hepatocytes (Fig. 5D). In high-dose of 5-MeO-MiPT, a serious increase in cells stained intensely Caspase-3 has been detected. The statistics of the results obtained from the H-score showed that the increase in the number of Caspase-3 positive cells was significant compared to the control (Fig. 11) (p<0.05). In kidney tissues, Caspase-3 positive cells were found in glomeruli. In vehicle control kidney section and low-dose of 5-MeO-MiPT, Caspase-3 staining appeared similar (Fig. 6B and 6C). In high-dose of 5-MeO-MiPT, especially in glomerular structure, intense Caspase-3 immunoreactivity was observed (Fig. 6D) and the increase in the number of Caspase-3 positive cells compared to the control was statistically significant (Fig. 11) (p<0.05). In brain section, there were few Caspase-3 positive cells characterized by a light brown staining pattern in high-dose of 5-MeO-MiPT (Fig. 7D). The statistics of the results obtained from the H-score showed that the rising number of Caspase-3 positive cells was significant compared to the control (Fig. 11) (p<0.0183). However, there was low level of Caspase-3 immunoreactivity in low-dose of 5-MeO-MiPT brain sections (Fig. 7C) and the increase in Caspase-3 immunoreactivity is not statistically significant (p>0.05).

Caspase-8 staining showed similar pattern to Caspase-3 and cytoplasmic areas were found to be stained within the cell. In liver, kidney and brain sections, there was no immunoreactivity of Caspase-8 in the observed control tissues as shown in Figure 8A, 9A, and 10A. In vehicle control liver section, there were few Caspase-8 positive cells characterized by a light brown appearance as shown in Figure 8B (p<0.017). The low dose of 5-MeO-MiPT group revealed a weak positivity of Caspase-8 in liver sections (Fig. 8C). In the high-dose 5-MeO-MiPT group, a rise in the number of medium and intense caspase-8 stained cells was detected. The numbers of positive cells at low and high doses of 5-MeO-MiPT group were statistically significant compared to the control (Fig. 11) (p<0.05). In the kidney tissue, except for the high-dose of 5-MeO-MiPT, there was no significant increase in Caspase-8 immunoreactivity (Fig. 9 and 11). In the high-dose 5-MeO-MiPT group, Caspase-8 immunoreactivity was detected in the glomerular structures (Fig. 9D). Compared to control, the increase in Caspase-8 immunoreactivity was found to be statistically significant (Fig. 11) (p<0.05). In Cerebellum sections, medium and weak Caspase-8 stained cells were detected in all and high doses of 5-MeO-MiPT groups (Fig. 10C and 10D).

**Toxicological results**

After pretreatment of blood and tissue samples, chromatographic method parameters and chromatogram for 5-MeO-MiPT parent drug are shown in Table 1, 2 and Figure 1. All biological samples were analyzed by using the method mentioned above. 5-MeO-MiPT results in all control and vehicle control samples were negative (under LOQ level). 5-MeO-MiPT, parent drug, was found negative (under LOQ level) in biological samples of 0.27 mg/kg dose mice. 2.7 mg/kg of body weight calculated and mice blood analysis results were found between 2.7-13.4 ng/ml. 5-MeO-MiPT was found 11-29 ng/g in the kidney, 15.2-108.3 ng/g in the liver, and 1.5-40.6 ng/g in the brain. Urine samples were collected with PBS and 5-MeO-MiPT were found under LOQ value. Since we do not have reference standards for metabolites of 5-MeO-MiPT, metabolites could not be detected in biological samples.

**DISCUSSION**

Today, almost every day a new NPS is produced, and these substances, which are illegal for both health and forensic medicine, pose a significant threat. The hallucinogenic tryptamine analog that we analyzed in our study, together with piperazine and phenethylamine derivatives, causes social problems worldwide (22). The identification of these substances, whether used intentionally or unintentionally, is imperative and important for forensic toxicologists. In order to evaluate these abusive substances, the substances and their metabolites are determined in human samples. It is absolutely necessary to elucidate the metabolic pathways of a new substance into the bazaar and to ensure accurate and reliable identification by selecting the appropriate analytical method. However, although there are several studies on the metabolism of hallucinogenic tryptamine analogs in humans, there are no studies in the literature on acute toxicity (13,22).

Even though hallucinogens are mostly evaluated as safe molecules, in recent years poisoning and death have been reported regarding the use of recreational tryptamines. Therefore, further research on the toxicology of synthetic tryptamines is needed to understand the true potential hazard (9). In recreational use of tryptamines, small amounts of intake are sufficient to produce psychotropic phenomena. However, it has been linked with poisoning and death for various reasons, including low toxic concentrations. These molecules cannot be routinely detected by typical screening panels, so tests, especially in emergency/clinical wards, result in false-negative, leading to inaccurate/limited assessment of illegal/controlled substances,
as well as inaccuracies in diagnosis and treatment, in the context of legal regulations. It is very important for experts to achieve information on these substances for public health and public safety (11). Furthermore, these compounds are difficult to detect in biological samples in order to determine the origin of poisoning or use. The main obstacle stays in the content of the target compound, which in most cases is not known by the analyst. In this study, as the expected control and vehicle control groups no 5-MeO-MiPT was detected. By given low doses group 5-MeO-MiPT was unfortunately not detectable by our analytical method. Compatible with our study, Fabregat-Safont et al. stated that 0.27 mg/kg IP injection, 5-MeO-MiPT was swiftly metabolized, being nearly completely removed in blood-stream after 60 min and transformed to many metabolites (20). However, high doses injected groups 5-MeO-MiPT distribution was shown in mice main organs kidney, liver, and brain. Since we do not have reference standards for metabolites of 5-MeO-MiPT, metabolites could not be detected in biological samples. We could not find any literature on the detection of 5-MeO-MiPT in organs.

Mice were used in this study to evaluate acute toxicity related to 5-MeO-MiPT, one of the tryptamine derivatives. Little is known about the human metabolism and toxicity of 5-MeO-MiPT in the assessment of potential harm to humans. Undoubtedly, the most accurate information can be obtained through studies on human beings, but human data is limited to individual poisoning cases. Ethical challenges in conducting clinical trials of these substances are restrictive in accessing this information. At this stage, in vitro experiments using microsomes or cell cultures and in vivo experiments animal models are most commonly used to generate a potential consumption marker (20).

Our study data showed that low-dose 5-MeO-MiPT did not cause any serious histopathological effects on the liver, kidney, and brain. High doses induce apoptotic cell death through caspase activity in some parts of the organs, especially glomeruli, central vein and portal areas. In this study, the effects on liver and kidney besides of the brain have been demonstrated. Studies on the analysis of the amount of NPS in serum and urine have been identified. Unfortunately, the literature on the effects of NPS exposure at organ level seems to be very limited. The effects of NPS on the brain have been shown in several studies (23-24).

In this study, it has been shown that high doses of 5-MeO-MiPT may cause some histopathological effects in different organs (brain, liver, and kidney). Furthermore, a high dose of 5-MeO-MiPT has been investigated to induce apoptotic cell death. The appearance of caspase activity at high doses in a short time is quite a surprise. The time between initiation of apoptosis to completion may last as short as 2-3 hours (25). Apoptosis plays a critical role in maintaining normal growth and hemostasis and regulating cell population balance in tissues (24). Normal tissues have caspase activity, even at low expression levels (27, 28). Therefore, we think that high doses of 5-MeO-MiPT accelerate the normal apoptotic process in the tissues. There are quite controversial results in the literature on the effects of tryptamine analogs. 5-MeO-DMT, one of the psychoactive tryptamines, has been shown to increase proliferation, survival, and maturation of neurons (26). However, there is data in the literature that it has negative effects on brain cells and triggers cell death. In a study by Herrera et al., tryptamine has been shown to trigger cell death in neuron and glia cells (23). In addition, one of the psychoactive tryptamines, 5-MeO-DiPT, has been shown to induce the development of oxidative stress, leading to neurotoxic effects (24).

Our study showed that 5-MeO-MiPT, especially at high doses, triggered apoptotic cell death from increased caspase-3 and caspase-8 activities. However, more detailed analyses are needed to fully elucidate the effects of the drug on cell death.

So far, by majority publications for 5-MeO-MiPT have concentrated on the profiles of receptor interaction, in vitro studies and case reports. Due to biotransformation of new psychoactive substances, produced metabolites cannot be easily, rapidly identified and absence of reference standards occur a general problem for laboratories that perform toxicological analysis. This study presents simple and basic histotoxicological information about 5-MeO-MiPT acute toxicity in an in-vivo mice model, which may lead to similar analysis in postmortem cases. We may not able to measure the metabolites 5-MeO-MiPT, this is our limitations for this study, but analyzing postmortem tissues substance levels beside blood and urine is important for forensic aspect (11).

References


<p>| TABLE 1. LC-MS/MS parameters for the MRM acquisition mode |</p>
<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time</th>
<th>Precursor Ion</th>
<th>Product Ions</th>
<th>CE</th>
<th>ESI</th>
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<tr>
<td>5-MeO-MiPT</td>
<td>11.4</td>
<td>246.80</td>
<td>86.10</td>
<td>-15</td>
<td>+</td>
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<td></td>
<td></td>
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<td>174.10</td>
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<td></td>
<td></td>
<td>159.10</td>
<td>-30</td>
<td>+</td>
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</tbody>
</table>

CE: Collision energy, ESI: Electrospray ionization mode

<p>| TABLE 2. The method parameters |</p>
<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time</th>
<th>Tissue R^2</th>
<th>LOD</th>
<th>LOQ</th>
<th>Blood R^2</th>
<th>LOD</th>
<th>LOQ</th>
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<td>5-MeO-MiPT</td>
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<td>0.997</td>
<td>0.3 ng/g</td>
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<td>1.5 ng/ml</td>
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FIG 1. 5-MeO-MiPT chromatogram and ions

FIG 2. The histological sections of mice liver stained by H&E. A) Control group, B) Vehicle control, C) 0.27 mg/kg 5-MeO-MiPT group, D) 2.7 mg/kg 5-MeO-MiPT H&E staining. Arrowhead indicates hepatocyte swelling, arrows point to immune cell infiltration. CV: central vein. Scale bar: 50 μm; inset scale bar, 20 μm).
FIG 3. The histological sections of mice kidney stained by H&E. A) Control group, B) Vehicle control, C) 0.27 mg/kg 5-MeO-MiPT group, D) 2.7 mg/kg 5-MeO-MiPT H&E staining. Arrows indicate Bowman’s Space. Renal structures indicated as glomerulus (G), proximal tubule (P), distal tubule (D). Scale bar: 50 μm; inset scale bar, 20 μm).

FIG 4. The histological sections of mice brain stained by H&E. A) Control group, B) Vehicle control, C) 0.27 mg/kg group, D) 2.7 mg/kg 5-MeO-MiPT H&E staining, Scale bar: 50 μm; inset scale bar, 20 μm)
FIG 5. Immunoreactivity of Caspase-3 in liver. A) Control group, B) Vehicle control, C) 0.27 mg/kg 5-MeO-MiPT group, D) 2.7 mg/kg 5-MeO-MiPT IHC staining. Brown staining represented by black arrows show caspase-3 positive cells. Scale bar: 50 μm; inset scale bar, 20 μm).

FIG 6. Immunoreactivity of Caspase-3 in kidney. A) Control group, B) Vehicle control, C) 0.27 mg/kg 5-MeO-MiPT group, D) 2.7 mg/kg 5-MeO-MiPT IHC staining. The areas within the black circles indicate caspase-3 positive cells in the glomerular structure. Scale bar: 50 μm; inset scale bar, 20 μm).
FIG 7. Immunoreactivity of Caspase-3 in brain. A) Control group, B) Vehicle control, C) 0.27 mg/kg 5-MeO-MiPT group, D) 2.7 mg/kg 5-MeO-MiPT IHC staining. Brown staining represented by black arrows indicate caspase-3 positive cells. Scale bar: 50 μm; inset scale bar, 20 μm).

FIG 8. Immunoreactivity of Caspase-8 in liver. A) Control group, B) Vehicle control, C) 0.27 mg/kg 5-MeO-MiPT group, D) 2.7 mg/kg 5-MeO-MiPT IHC staining. Scale bar: 50 μm; inset scale bar, 20 μm).

FIG 9. Immunoreactivity of Caspase-8 in kidney. A) Control group, B) Vehicle control, C) 0.27 mg/kg 5-MeO-MiPT group, D) 2.7 mg/kg 5-MeO-MiPT IHC staining. The areas within the black circles indicate caspase-8 positive cells in the glomerular structure. Scale bar: 50 μm; inset scale bar, 20 μm).
FIG. 10. Immunoreactivity of Caspase-8 in brain. A) Control group, B) Vehicle control, C) 0.27 mg/kg 5-MeO-MiPT group, D) 2.7 mg/kg 5-MeO-MiPT IHC staining. Scale bar: 50 μm; inset scale bar, 20 μm).