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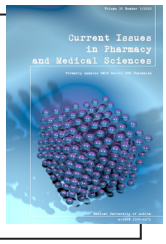

Mephedrone – a synthetic derivative of cathinone

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ABSTRACT

Novel psychoactive substances (NPS) are a very diverse group of recreational drugs that mimic effects of classic drugs of abuse and, at least at the beginning, are not usually prohibited. Representative of this group is mephedrone – a cathinone derivative. It is widely used as a recreational drug, particularly among club guests. Mephedrone`s effects are compared to effects of cocaine and MDMA but are more short-lived and include: psychostimulation, enhanced empathy, reduced feeling of tiredness, euphoria, hallucinations. This drug also exerts adverse effects, such as: anxiety, delusions, paranoia, psychosis, increased body temperature, elevated blood pressure, sleep disturbances. According to surveys performed on mephedrone users, it may be addictive. Those findings confirms a growing number of behavioural and molecular studies on animals. Mephedrone acts mainly via increasing monoamine transmission through increasing release of dopamine, serotonin and noradrenaline into synaptic cleft, inhibiting their re-uptake and reducing their metabolism. However, participation of other transmitters, modulators and pathways are investigated, including glutamate and nitric oxide. Favorable routes of administering mephedrone is intranasal and per os. Moreover, most often drug users use it in a binge way, e.g. taking repeated doses of a drug in a short period of time. According to animal studies, this pattern of mephedrone use leaves more neural injuries than taking it regularly, but in smaller doses. Our aim was to present a short, but essential, overview of the current knowledge on mephedrone, focusing on its effects, mechanism of action, animal studies evaluating its influence on the brain structures, toxicity and pharmacokinetics.

INTRODUCTION

Natural derivatives of cathinone (S - (-) - 2-amino-1-phenylpropane-1one) are alkaloids found in the evergreen shrub from Ethiopia called Khat, i.e. Catha edulis. Due to its stimulating properties, khat was in use in antiquity. Indeed, the ancient Egyptians called it as the "divine food". For inhabitants of East Africa and the Arabian Peninsula, it is a "ceremonial drug", which is consumed as a brew, or is smoked or chewed during religious ceremonies and social gatherings. To this day, new leaves of this plant are also used in folk medicine, in the treatment of depression, asthma, and in order to eliminate the feeling of hunger and fatigue [1,2]. The main representative of the group is cathinone, which in mature plants is metabolized into cathine (1S, 2S-pseudoephedrine), a substance with weaker potential of action [3]. Synthetic cathinone derivatives are structural analogs of this natural compound, distinguished by the presence of a ketone group in the β position of the side chain [4]. They are obtained in laboratory settings by replacing the hydrogen atoms with CH3 groups, C2H5 groups, or methoxy groups, and with chlorine, bromine or fluorine. Main representatives of this group are: mephedrone (4-methylmethcathinone), 3,4-methylenedioxypyrovalerone (MDPV) and naphyrone (naphyropyrovalerone) [5]. By the end of 2021, the European Monitoring Centre for Drugs and Drug Addiction (EMCDAA) had listed over 162 different synthetic cathinones. This number makes this group of active agents (after synthetic cannabinoids), the second largest group of new psychoactive substances (NPS). In our review we will focus on mephedrone, a popular cathinone derivative that belongs to the NPS group.

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1. KHAT

Khat (Latin *Catha edulis*, Celastraceae family) is a shrub with large, shiny and "evergreen" leaves. Cultivation of khat is widespread in countries bordering the Red Sea (Yemen, Sudan, Eritrea, Saudi Arabia), as well as in Ethiopia, Somalia, South Africa, Kenya and Madagascar. For centuries, in these areas, khat was treated as the "divine food" [1], as parts of this plant were chewed for medicinal, recreational and religious purposes. Chewing fresh shoots of the khat, often for many hours, releases numerous active plant ingredients such as flavonoids, tannins, glycosides, sterols, vitamins and minerals, as well as psychoactive alkaloids (cathinone, cathine) [1,6]. Cathinone (S (-) - α-aminopropiophenone) is structurally similar to amphetamine with a β-keto group [4,7], and when delivered to the body, it breaks down into cathine and norephedrine, and consequently, it produces an analogous psychotropic effect to amphetamine.

Khat consumption causes euphoria, stimulates and increases talkativeness and unconventional thinking, improves concentration and enhances self-confidence. At the same time, studies show that taking khat leads to a number of side effects. Nausea, abdominal pain and dizziness, for example, have been observed immediately after administration. What is more, long-term use of this stimulant results in, among others, cognitive and cardiovascular disorders (hypertension, tachycardia, myocardial infarction), abnormalities in the gastrointestinal tract (gastric and duodenal ulcers, hemorrhoids, constipation) or the genitourinary system (problems with urination, impaired fertility). Additionally, in the area of the oral cavity, gingivitis, tooth decay, and even dental cavities have been observed [1,8]. Some data also indicate an increased risk of the development of esophageal [9] and stomach cancer in khat users [8]. Moreover, among people taking khat, a rapid development of psychological dependence has been noticed, even after the use of small doses [10]. After discontinuation of khat, users have experienced withdrawal symptoms, including sleep problems, irritability, confusion, as well as mood disorders and emotional lability [11]. A review of the literature shows that recently, with the influx of African emigrants, the use of this plant has become more and more popular in Europe, as well as in the USA and Australia [12]. At the same time, the use of extracts from khat in designed NPS has emerged. According to current literature, 118 new synthetic cathinone derivatives have been identified since 2004 [13].

2. MEPHEDRONE

Basic information

Mephedrone [IUPAC: (RS)-2-methylamino-1-(4-methylphenyl)propan-1-one; 4-MMC, 4-methylmeth-cathinone] is a semi-synthetic cathinone derivative, substituted β-keto-amphetamine, and is one of the most popularly consumed novel psychoactive substances (NPS). NPS are substances newly introduced to the market, starting from circa 2005, that mimic the effects of several illegal drugs of abuse. They are a diverse group of psychotropic or narcotic substances in terms of origin, action and chemical structure. Firstly, these are deemed "legal highs", because most legal, but with legislation changes, they are gradually being listed as banned substances [14]. Mephedrone is often referred to as a "natural amphetamine" due to its commonly known psychostimulatory properties [15]. It is the main ingredient in the blends of designer drugs known as "bath salts" or "plant nutrients", in slang it is also called "Meow Meow", "4-MMC", "Meph", "Drone", "M-cat", "Rush", "Bubbles", "Drone", "MMC Hammer", "Diablo XXX" [15,16]. Mephedrone production requires similar laboratory equipment and know-how as the synthesis of amphetamines. There are two ways of manufacturing mephedrone. The first has two steps: bromation of 4-methylpropiophenone and reaction with metylamine. The second way to produce mephedrone is via the reaction of oxidation of 4-methylephedrine by $KMnO_4$ [17]. Mephedrone was for the first time synthesized and described by Saem de Burnag in 1929, in France [18]. After many years, at the turn of the 20th and 21st centuries, the psychotropic effect of mephedrone was discovered, thanks to which it again gained interest among scientists. Due to this feature, the substance also became the subject of widespread drug trafficking [15]. According to literature, the first time that mephedrone was used as a drug happened in Israel at the beginning of the 21st century [4]. Mephedrone production flourished again by 2003, and it reached the European market in 2007. By mid-2009, its presence was well-established in the British market, and in 2010, mephedrone was listed at fourth place among the most popular drugs according to the European Monitoring Center for Drugs and Drug Addiction. In the UK, it was a very popular 'party' drug, consumed in various types of nightclubs and discos, gaining most popularity among 15-24-year-olds [15,16]. Competitive price, widespread availability in online stores and a low toxicity potential have made mephedrone a replacement for MDMA [19,20] Many countries, including UK, banned mephedrone, but this did not reduce interest in the drug – on the contrary. A survey carried out amongst club guests showed that in 2010, 27% of all club-goers wanted to use or used mephedrone. By 2011, this number increased to 41% [21].Currently, mephedrone is used as a stimulant and empathogen, mainly by adolescents for recreational purposes. According to the report by the European Monitoring Center for Drugs and Drug Addiction [13], in the UK in 2015 and 2016, mephedrone was used by 0.5% of all young people. Moreover, mephedrone has become popular among people addicted to psychoactive substances (mainly amphetamine and its derivatives), opioid users and prisoners [22]. Some data suggests that mephedrone popularity in the UK has, however, dropped. EMCDAA [23] has shown that mephedrone use significantly diminished between 2014 and 2018 amongst 16-34-year-olds (i.e., 1.1% versus 0.0%). Still, the same report mentioned, that 3 laboratories producing mephedrone were discovered (Poland, Spain, Netherlands) in Europe in 2018, and that 50 kilograms of cathinone substrate (2-bromo-4-methylpropiophenone) was confiscated, which indicates that there was still a market demand for manufacturing this drug. Moreover, data collected in the UK from the Toxicology Unit, Imperial College London [24], showed that in 2014, mephedrone was discovered in 1% of all samples collected

post-mortem (samples from the coroner working in London and in a part of England), and in 2015, this number rose to 1.5 %. There are also data collected between 2010 and 2018 at the Nowowiejski`s Hospital in Warsaw, in Poland that suggest that in the recent past, mephedrone has retained its popularity as a clubber's drug of choice. In the above-mentioned years, 601 people needed professional help in this hospital after using mephedrone in a binge manner (at least consecutive 2 days of using mephedrone). Herein, 93% were men, 55% of patients were 26-35 years old and almost all of them (99%) used the drug with another substance of abuse [25]. Mephedrone is available in a solid state as hydrochloride or sulfate, and is sold as a white powder, or as capsules or tablets. It is easily soluble in water. Its free base is a yellowish liquid. The powder is a racemic mixture of enantiomers [26]. The most common routes of administration are inhalation and intranasal use, as the oral bioavailability of this drug is only 10% [27]. Mephedrone is also sometimes taken in large, single doses by swallowing a substance wrapped in cigarette paper ("bombing"). Due to its good solubility in water it is possible to administer this agent intramuscularly, subcutaneously, as well as in a form of rectal suppositories or intravenous injections (so-called "slamming") [15]. The intravenous route of administering mephedrone in combination with other substances, e.g. methamphetamine, γ-hydroxybutyrate acid cocaine or sildenafil in order to obtain sexual arousal lasting for several hours, is gaining popularity especially among MSM (men having sex with men) [22,24]. In a survey performed in a population of 145 patients 84.4% preferred an intranasal application of mephedrone, and 11% preferred an intravenous route [28]. The timing and onset of action of mephedrone depend on the route of administration. As shown by studies in rats, after intravenous administration, the maximum concentration in the brain is reached after 2 min, intranasally after about 30 min, and after oral administration – from 15 to 45 min [29,30]. Typical intranasal doses for mephedrone users are 25-75 mg, while oral dosages are higher, 150-250 mg. Users report that after ingesting mephedrone, its effects start in 15-45 min and last for 2-4 h. After self-administration of mephedrone via the nasal route it starts working in 10-20 min, and the effect lasts for 1-2 h. An intravenous route gives first effects after 10-15 min, but their duration is the shortest amongst the above-mentioned – this lasts for 30 min [16]. An oral administration of mephedrone, besides longer duration of action, is also characterized by weaker side effects and a lower addiction potential [15]. According to mephedrone users, a typical "session" lasts about 10 hours and during this time the user takes 0.5-1 g of the drug divided into several doses. Some people take mephedrone at first intranasally during one session, and then orally, in order to achieve both a quick and delayed effect [15,31]. Regular, heavy users may administer even 16 g of mephedrone during one session, but the mode is 1 g [17]. As a psychotropic substance with an addictive potential, mephedrone is not used in pharmacotherapy [32]. Since 2010, mephedrone has become a controlled compound in numerous European countries [33]. Precautions were introduced due to the growing number of people hospitalized after administration of this substance and after reports of deaths as a result of its overdose [34]. As a consequence of the more and more disturbing data related to the use of mephedrone in the USA, the then President Barack Obama signed an act banning this substance [15,35]. In the European Union, the Council of UE decided to introduce mephedrone to the list of controlled substances around the same time [36]. As a result, European countries have had to introduce a number of legislative changes leading to the banning of mephedrone. In Poland, the change in law was introduced on June 10th of 2010 and since then mephedrone has been allowed only for scientific purposes (it belongs to the so-called group 1-P of psycho-tropic substances) [37]. As in many other countries, in the UK, it is also under control, as it is classified since April 2010 as a class B substance under the Misuse of Drugs Act [38].

Mechanism of action

Due to its high lipophilicity, mephedrone has an ability to cross the blood-brain barrier [30]. Mephedrone exerts its effects by changing levels of certain monoamines in the central nervous system (CNS) and peripherally in several ways. Scientific literature provides much data collected from animal research.

A range of studies has shown that similarly to other synthetic cathinones or amphetamines, mephedrone inhibits the re-uptake of neurotransmitters (dopamine, norepinephrine, serotonine), and therefore their transport through cell membranes back to the cytoplasm of nerve cells by interacting with the plasma membrane monoamine transporters [29,35,39-43]. This activity is, among others, due to the blockage of biogenic amine transporters by mephedrone, which is treated by the transporters like a substrate [42,44-46]. Researchers have shown that mephedrone inhibits with a significant potential the norepinephrine transporter (NET) in human embryonic kidney (HEK) 293 cells [44]. Importantly, the potential with which a given psychostimulant (also cathinones) blocks NET correlates with doses administered in humans [47]. Moreover, norepinephrine plays a crucial role in the acute effects of psychostimulants, and its brain levels correlates with doses self-administered by humans [48]. Luethi *et al.* [44] also demonstrated that mephedrone blocks the dopamine transporter (DAT) and serotonine transporter (SERT) with similar strength. This outcome is in line with findings of other authors [Baumann, 2012. Hadlock *et al.,* 2011; Simmler *et al.,* 2013]. However, there are some papers indicating that mephedrone blocks DAT more profoundly than it blocks SERT [41,42,45,46]. It seems that in a case of mephedrone, transporter selectivity is not clearly established – Luethi *et al.* [44], for example, has shown that mephedrone is more selective to NET than to DAT, but other authors [42,45,46] have not observe such a marked selectivity. Still, Hadlock *et al*. [40] revealed that mephedrone administered to rats in a binge-mimicking way inhibits the activity of DAT in the stratium and SERT in the hippocampus while increasing the release of both DA and serotonine (5-HT) into the synaptic cleft. In contrast, some studies have shown that frequent but short-term administration of mephedrone did not cause a permanent depletion of catecholamines in the cortical structures of the brain and striatum, unlike MDMA (35), while other studies have

demonstrated that mephedrone increases 5-HT and DA levels in the nucleus accumbens [49,50] striatum, and frontal cortex in rats [50]. Another component of the mechanism of action of mephedrone is related to an enhanced efflux of monoamines to the synaptic cleft. In experiments by Motbey *et al.* [51], rats were given intraperitoneally mephedrone at a dose of 30 mg/kg (i.e., the dose corresponding to 200-300 mg administered to people) and 1 hour after the injection, levels of monoamines were evaluated. In the striatum, a significant increase in DA concentration was observed and a decrease in levels of this monoamine metabolite. In contrast, 5-HT levels in the striatum and hippocampus were lowered, and the levels of serotonin metabolites were increased. Furthermore, this effect was time-dependent. The highest level of serotonin was detected 20 min after the injection [49], and the highest level of dopamine was obtained 40 min after the injection. Therefore, 1h after drug administration, serotonin could have been already metabolized to a large extent. These findings are in line with behavioural responses of mephedrone users – mephedrone gives them a rapid high, but this effect is rather short-lived, which predisposes to re-administration of the drug [51]. Kehr *et al.* [49] showed a significant elevation of DA levels in the nucelus accumbens in rats after meophedrone treatment, for example, which was similar to the effect observed after amphetamine, and similarly to MDMA, mephedrone also caused significant changes in 5-HT levels. These outcomes were in line with findings obtained by Aarde *et al.* [52] and Baumann *et al.* [39], who demonstrated that administration of mephedrone resulted in elevation in dopamine and serotonin extracellular concentrations, and this effect was more profound for serotonin.

A few experiments have been performed to evaluate long term effects on monoamine levels after mephedrone treatment. Motbey *et al*. [51] showed that 7 weeks after a repeated dose of mephedrone, there is no change in DA and 5-HT levels in rat brains. Similarly, Bauman *et al.* [39] and den Holleander *et al.* [53] revealed that 14 days after discontinuation of mephedrone treatment (binge manner – 1 day and binge manner – 4 days, respectively), mephedrone had little or no influence on DA, 5-HT and NE levels. However, Hadlock *et al.* [40] observed that 7 days after the last injection of mephedrone (binge-like, 1 day), 5-HT levels in the hippocampus were still diminished. Such an effect was not observed when DA levels were tested. It should be mentioned that both effects were similar to the ones observed after MDMA administration.

In line with these findings, Martinez-Clemente *et al*. [54] revealed that 7 days after a specific mephedrone treatment – 4 mephedrone doses of 50 mg/kg per day (which is equivalent to 1.5 g in humans in one session), levels of DAT were reduced in the striatum and cortex, whereas levels of SERT were diminished in the hippocampus. In experiments performed in the same way, but with the use of a lower mephedrone dose (25 mg/kg, 4 times a day), DAT levels were decreased only in the cortex, but no changes in DAT concentrations were detected in the striatum and hippocampus. A schedule that mimicked a weekend, binge use (i.e., 3 times a day, 25 mg/kg, for two days) caused the greatest neurological changes, including a significant decrease in DAT and SERT levels in the cortex and hippocampus alongside with astriogliosis. These data indicate that mephedrone used in a binge-like way may exert neurotoxic effects. On the other hand, Angoa-Perez *et al.* [55] showed that mephedrone did not stimulate astrocytes or microglia in the striatum and prefrontal cortex.

These discrepancies in study outcomes may be due to differences in study design – i.e., experiments were carried out in different rodent species, with different schedules of drug administration, as well as different time interval between the last administration of the drug and brain tests. Therefore, there is a need for further research in order to better understand the mechanism of action of mephedrone and long-term effects of its use. Nevertheless, it seems that higher doses of mephedrone administered in a short time and in a prolonged time produce a short-lived neuronal toxicity towards the serotoninergic and dopaminergic system in the brain. Neurotoxicity of mephedrone was also demonstrated in pups born to mothers who were administered mephedrone during pregnancy. Pups presented hippocampal toxicity and some specific behavioural responses [56] (Table 1).

Mephedrone, moreover, also displays affinity for 5-HT2A, 5-HT2C, 5-HT1A, alpha-1A adrenergic *receptor* (α1A), alpha-2A adrenergic *receptor* (α2A), and trace amine-associated *receptors* (TAAR) [44,45,57,58]. There is also evidence that mephedrone interplays with the glutamatergic neurotransmission [59-62].

There are some findings on mephedrone`s cytotoxicity caused by oxidative stress. In addition, it seems that the drug causes alteration in the respiratory chain in mitochondria in the brain tissue [63]. Indeed, studies by Budzyńska *et al*. [64] showed some changes in oxidative stress markers in the prefrontal cotex and hippocampus in mice, i.e. lowered levels of antioxidants, diminished activity of catalase (which indicates reactive oxygen species (ROS) overproduction) and finally, significantly elevated levels of malondialdehyde.

Pharmacokinetics

Currently, there is little data regarding the pharmacokinetics of mephedrone. The data obtained from tests carried out in human volunteers who ingested 200 mg of mephedrone indicated that the maximum plasma concentration of mephedrone is achieved 1 h and 15 min later, with Cmax of 134.6 ng/ml. It was also demonstrated that the half-life of mephedrone is 2 h and 9 min, which is significantly lower than the half-time of amphetamine (t1/2=10-12h), MDMA (t1/2=8h) or methamphetamine (t1/2=15h). The drug was undetectable after 24 h [34]. Other human-based studies carried out in 6 healthy men showed that after intranasal administration of mephedrone at a dose of 100 mg, C_{max} in plasma was 89.8±41.7 ng/ml with T_{max} = 55 min (± 18.2 min) [65] Olesti *et al.* [66] performed tests in healthy men using a range of mephedrone doses administered orally (50, 100, 150, 200 mg). The dose of 100 mg resulted in C_{max} of 51.7±20.5 ng/ml with T_{max} of 60 min. These findings confirmed that the intranasal administration of mephedrone used in similar doses produces effects more quickly with a higher level of maximal concentration. This is in line with reports from mephedrone users that insufflation of the drug produces quicker and more intensive sensations.

Table 1. Effects of mephedrone on animals

Animal species	Mephedrone treatment schedule	Behavioural effect	Author
Female Balb/C mice, pups born from mephedrone-treated mothers	Pregnant female 50 mg/kg mephedrone one time a day 5-18 day of gestation	Increased water intake, decreased body weight gained during pregnancy, low birth weight of pups, higher stillbirth rate, weakened spatial learning and reference memory	[56]
	Pregnant female 50 mg/kg mephedrone three times after 5, 6, 11, 12, 17, 18 days of gestation		
Female C57BL/6 mice	Binge-like 20 mg/kg 4 times, 2 hours break between each injection	Increased body temperature, increased locomotor activity	[101]
	Binge-like 40 mg/kg 4 times, 2 hours break between each injection	After 2nd injection - hypothermia and then hyperthermia; increased locomotor activity	
Male Wistar rats	One-time administration of 15 or 30 mg/kg	Increased locomotor activity, lowered social preference, activation of VTA and nucelus accumbens (striatum)	[102]
Male Sprague-Dawley rats	0.5, 1, 3, 5, 10, 30 mg/kg – acute	Doses 3-30 mg caused increased locomotor activity	[103]
	0.5 mg/kg once daily for 5 days, 10 day break and one dose of 0,5 mg/kg of mephedrone	Sensitization to low dose of mephedrone	
	1 injection a day of 3, 10 or 30 mg/kg of mephedrone, for 4 days	Produced conditioned place preference	
CD-1 mice	30 mg/kg once a day, 6 days	Produced conditioned place preference	
Male Lister hooded rats	Mephedrone 1, 4 mg/kg or 10 mg/kg for 2 consecutive days a week, 3 weeks – binge	All doses increased locomotor activity; after 6th dose of 10 mg/kg – sensitization to locomotor activity; impairment of novel object discrimination;	[43]
Male rhesus monkeys	acute doses of 0.178, 0.32, 0.42, 0.56 mg/kg	Improvement in visuospatial associative memory	[104]
Adult Swiss CD-1 mice	5, 10, 25 mg/kg – acute	Increased locomotor activity	[29]
Male ICR mice	3, 10, 30 mg/kg – acute	Increased locomotor activity	[105]
	10, 30, 56 mg/kg – acute	FOB: increased activity, overstimulation, stereotyped head weaving, head circling Rotarod: no effect	
Male Sprague-Dawley rats	1 or 3 mg/kg – acute	Increased locomotor activity	[49]
Male Sprague-Dawley rats	15 mg/kg - first day, 30 mg/kg for 5 days, 15 mg/kg on the last day, 2 and 10 day abstinence, then challenge – 15 mg/kg	Sensitization to repetitive movements	[106]
	15 mg/kg for 5 days – 10 day abstinence, then challenge – 15 mg/kg		
Male C57BL/J6 mice	30 mg/kg twice daily for 4 consecutive days – binge;	Impairment of working memory – examination 3 weeks after last injection, Increase in body temperature – temperature measured 45 minutes after each injection	[53]
Male adolescent Wistar rats	30 mg/kg – acute	Increased locomotor activity	[51]
	30 mg/kg for 10 days	Increased locomotor activity, decreased weight gain	
	7,5, 15, 30 mg/kg once a day for 10 days	30 mg/kg – deficit in memory – NOR test taken 35 days after last injection	
Planarians	a solution of mephedrone: 0, 10, 50, 250, 100, 500, 1000 μM in petri dish	Stereotyped behaviours	[107]
	1 and 10 μM in petri dish pretreatment and then discontinuation of mephedrone treatment	Lower motility – i.e. withdrawal symptoms	
	10, 100, 500 μM in petri dish for 30 min in the non-preferred environment	Conditioned place preference to mephedrone environment (dose 100 and 500 μM)	
Male C57BL/6J mice	1, 3, 10 mg/kg – acute	Reduces intracranial self-stimulation threshold in mice	[108]
Male Wistar rats; Sprague-Dawley rats	0.5 mg/kg/infusion – acute	Supports self-administration behavior; Wistar rats elicit higher response than Sprague-Dawley rats	[52]
Male Sprague-Dawley rats	0.03, 0.1, 0.3 and 1 mg/kg/infusion	Supports self-administration behavior	[109]
Male Swiss Webster mice	0.3, 1, 3, 10, or 30 mg/kg – acute	3 and 5 mg/kg – increased locomotor activity	[110]
Male Sprague–Dawley rats	0.5-5 mg/kg – acute	Substitutes for the discriminative stimulus effects of cocaine and methamphetamine	
Male albino Swiss mice	0.05, 0.125, 0.25, 0.5, 1, 2.5 or 5 mg/kg – acute	Increased locomotor activity	[111]
	0.05, 0.125, 0.25, 0.5, 1 or 2 mg/kg – acute	Dose 1 and 2 mg/kg – anxiolytic potential	
	0.05, 0.125, 0.25, 0.5, 1 mg/kg – acute	No change on rota-rod and chimney test	
	5 mg/kg for 7 days	Development of tolerance to hyperlocomotion	
	1.25, 2.5 and 5 mg/kg – acute	No proconvulsant, no anticonvulsant properties	
	0.125, 0.25, 0.5 or 1 mg/kg – acute	Improvement in spatial memory	
Male Wistar rats	0,5 mg/kg/infusion	Supports self-administration behavior	[112]

The metabolism of mephedrone is mediated by cytochrome P450 enzymes, mainly CYP2D6, and to a small degree by NADPH-mediated enzymes [67] and to a lesser extent, CYP3A4 [68]. There are few known metabolites of mephedrone. Among these are: nor-mephedrone, dihydro-mephedrone, 4-carboxy-mephedrone and succinyl-nor-mephedrone. Nor-mephedrone is likely to cross the brain-blood barrier and it is probably co-responsible for mephedrone`s effects [69]. Poor metabolizers are more vulnerable to side effects, and toxicity of mephedrone as the drug exhibits the 1st order kinetics – plasma levels of mephedrone and its effects increase when the used dose is being increased. Depending on an administered dose, 5-15% of unmetabolized mephedrone is excreted with urine [66].

Effects and health risks in humans

The literature provides a wealth of data on the effects of mephedrone, mainly from retrospective survey studies, but also, to a small extent, from controlled human trials. Effects of mephedrone are described by its users as comparable to amphetamines, cocaine and MDMA, but most participants of the study by Carhart-Harris *et al.* [31] seemed

to have chosen mephedrone effects being more akin to those of MDMA. Reported desired/positive effects of mephedrone include: intense stimulation, increased alertness, euphoria, empathy, increased confidence, talkativeness and openness. There were also increased sensory experiences and slight sexual arousal, as well as a change in the perception of time [15,32,70,71]. Van Hout and Brennan conducted a study involving 22 mephedrone users, interviewing them about their sexuality under the influence of mephedrone. Results showed an intensified sex drive, lack of inhibitions resulting in licentiousness, risky behaviours like intercourse with strangers, drug-sex sessions (chemsex) with many partners, and lack of protection against infectious diseases and pregnancy [72] Limited case reports shows several cases of MSM (men having sex with men), who due to mephedrone use acquired HIV and HCV and staphylococcal infections [73]. Comparing mephedrone to cocaine, most users reported longer activity, better "fly-off", and lower addiction potential of the former substance than the latter [70]. These desired effects were confirmed in human-based controlled study in which volunteers took 200 mg of mephedrone orally. Drug effects were described as similar to effects of MDMA (100 mg), but more short-lived [34].

Mephedrone also causes adverse effects. Psychopathological symptoms include psychosis, distorted perception of reality, paranoia, hallucinations, delusions, aggression, confusion, anxiety, panic, anhedonia and even suicidal thoughts [71]. From 2010 to 2018, in Warsaw`s psychiatric hospital 24,5% of all patients admitted because of mephedrone intake were noted to have attempted suicide, while 15,5% had self-harmed themselves. This is very alarming because the suicide attempts levels grew every year of this retrospective study [25]. The literature also indicates the possibility of neurological effects: pain and dizziness, convulsions, or sleep disturbances [74]. Teeth grinding, trismus and nystagmus have also been reported. Some data also indicate that mephedrone has the ability to interfere with peripheral thermoregulation [70]. Changes in the regulation of body temperature can be manifested by hot flushes and increased sweating (mephedrone sweat). Moreover, gastrointestinal effects (nausea, vomiting, loss of appetite), disturbances in genitourinary (difficulties with micturition, anorgasmia) and immune systems were observed [15].

Mephedrone intake has been known to induce cardiovascular stimulation manifested as elevated blood pressure, tachycardia and chest pain [34]. In addition, renal failure and rhabdomyolysis have been noticed [75]. Blue fingers and toes are considered as distinctive clues to facilitate identification of a mephedrone user [70]. A life-threatening side effect resulting from chronic administration of mephedrone or administration of this substance at high doses, is called 'sympathomimetic toxidrome', i.e. a set of symptoms resulting from a strong stimulation of the sympathetic system. Such adverse symptoms include cardiac disorders, hypertension, chest pain, blurred vision, pupil dilation and agitation [76]. Some, but limited, human observational studies/clinical studies confirmed the results of the above-mentioned retrospective questionnaire studies, case reports and reviews [34,77,78]. It should be noted that one of the side effects observed after chronic mephedrone use is Parkinsonism, resulting from manganese poisoning – an auxiliary substance used in home production of psychostimulants from ephedrine or pseudoephedrine [20]. Reports on the use of mephedrone in people with diabetes indicate that it can lead to ketoacidosis [79].

Additional side effects of mephedrone are related to its route of administration. Administration via nasal mucosa is associated with severe irritation and pain. In contrast, intravenous use of mephedrone causes a burning sensation, and multiple injections often block veins, cause local infections, abscesses, scabs, sores, blood clots, and other tissue abnormalities at the injection site. Moreover, the use of the same needles among addicts poses a risk of HIV infection [32,80]. Literature provides data on an increased incidence of injectable mephedrone use among HIV-positive, HCV-positive users, as well as a history of mephedrone overdose over the past year [80]. Another risk connected to mephedrone use is developing hyperthermia and serotonin syndrome [81].

The first recorded death, the sole cause of which was mephedrone abuse, was the death of an 18-year-old woman in 2008 in Sweden [16]. In fact, several case reports on mephedrone cause/related deaths can be found in medical literature [82,83]. Examples of this include a case of death from acute aortic dissection in a 29-year old mephedrone-dependent [84], and a case of death linked to mephedrone overuse caused by excitement delirium (hyperactivity, aggression, delirium) [85]. Schifano *et al.* [86] in 2012, performed an analysis of 128 deaths connected to mephedrone use. Accordingly, only 90 cases could truly be considered mephedrone-related due to mephedrone identification in tissue samples. Moreover, only 8 cases were caused by mephedrone only – the rest were because of a combination of mephedrone and other drugs. One death related to mephedrone and heroin use was also note) [87]. Loi *et al*. [88] also analyzed mephedrone-related deaths among 16-24 years old people. Researchers extracted 30 mephedrone-related deaths from UK coroners reports from 2009 to 2013. However, they indicated that the risk of death after mephedrone is not high, but clearly it increases as a result of combining mephedrone with alcohol or other psychoactive substances.

The effects of combining multiple drugs, including mephedrone, at once are unpredictable and treating their side effects is difficult. Some known interaction between mephedrone and other substances are presented in section Drug interactions.

Cases of mephedrone dependence and symptoms of "drug craving" among its users have been described in the medical literature [19,83,89]. A study conducted in mephedrone users showed that as many as 29.5% of all respondents had at least 3 symptoms of dependence according to DSM-IV. The predominant symptoms were: increased tolerance to the drug, impaired control of its use, continued use despite severe psychological and physical problems, and a strong desire to take another dose [74]. An Internet survey with 1506 volunteers showed that more than half of them experienced symptoms of drug abuse [31]. Another study, performed with over 1000 participants, demonstrated that 17.6% of them showed signs of addiction [90]. According to collective reports of mephedrone users and data reported from emergency

departments, withdrawal syndrome after using this substance is characterized by a lack of appetite or increased appetite, anxiety, depression, irritability and sleep problems [34,71,91]. Human studies indicate that taking concurrently alcohol and mephedrone increases the risk of addiction to the cathinone [92]. Moreover, it seems that the parenteral route is related with a higher risk of developing addiction [28]. Tolerance to the stimulating effect of mephedrone develops very quickly, therefore addicts consume more and more amounts of the drug in a short time, reaching even 1-4 g of the compound during one session [34].

Drug combinations

Literature data indicate that a common practice among mephedrone users is taking this substance, like other "legal highs", with other psychostimulants, hypnotics and psychedelic drugs, such as amphetamines, heroin, cannabinoids, cocaine, benzylpiperazine or ketamine [15,93]. This cathinone is also often taken together with alcohol [22]. To date, little is known about interactions of mephedrone with these substances. Outcomes of a clinical trial phase I confirmed that mephedrone taken together with alcohol partially abolished sedation induced by alcohol [77]. Further studies confirmed that mephedrone limits alcohol-caused sedation and feeling drunk and showed that co-administration of both drugs caused more severe cardiovascular effects and more pronounced feeling of euphoria than both substances taken separately. Moreover, it seems that the tendency to abuse mephedrone used concurrent with alcohol is higher than that caused by mephedrone alone [92,94]. Co-administration of mephedrone and alcohol also increases the amounts of alcohol consumed [95]. Taken together, combining mephedrone and alcohol poses a higher risk of intoxication and dependence developmen [92,94,95].

Animal studies have also shown that the combination of mephedrone with methamphetamine or MDMA, increases the neurotoxic effect on dopamine neuron terminals, while mephedrone itself did not show such an activity [55]. Budzyńska *et al.* [96] for example, tested behavioural effects of concurrent administration of mephedrone and MDMA and revealed that co-administration of the two drugs exerted antidepressive effects in EPM (elevated plus maze) test and amelioration of memory consolidation in PA test (passive avoidance). However, FST (forced swim test) showed no effect on anxiety. An interaction between mephedrone and caffeine or nicotine has also been described. Concurrent administration of the cathinone and caffeine induced hyperthermia [33], whereas coadministration of mephedrone and nicotine enhanced oxidative stress [64]. In addition, research regarding interaction between mephedrone and amphetamine showed that concurrent use of both substances exerted stronger sensitization to locomotor activity in mice [97]. A study conducted on 601 patients admitted to psychiatric hospital due to mephedrone regular use between 2010 and 2018 showed a relationship between the use of mephedrone with other substances and an increased risk of psychiatric complications. Intake of combination of mephedrone with alcohol or cannabinoids or opioids, for example, cause insomnia. A risk of suicide attempt also grew when mephedrone was taken together with another abusive substance [25].

There is little data concerning mephedrone (or cathinones in general) interaction with prescription drugs, we can, however, assume that they are present. ADHD drugs and antidepressants mechanisms of action are similar to that of cathinone, i.e. they elevate levels of monoamines in the synaptic cleft, hence, it is possible that using concurrently mephedrone and one of the mentioned drugs might result in additive effect and cause some severe consequences. Among these is the possibility of developing serotonin syndrome. This may even lead to death (more information about serotonin syndrome below). In particular, we can expect an interaction between venlafaxine and mephedrone, because both are mainly metabolized by CYP2D6. This can cause elongation of half-life and elevation of concentrations of both substances [98].

Literature also points that mephedrone interacts with bupropion, fluoxetine, paroxetine and terbinafine. These are inhibitors of the CYP2D6 enzyme [54]. A possible interaction comes about when using mephedrone alongside with cobicistat, an anti-HIV drug. This drug is an CYP2D6 inhibitor, therefore, co-use might elevate mephedrone levels and enhance its toxicity. Moreover, mephedrone can interact with another anti-HIV agent, ritonavir, as it inhibits CYP3A4 cytochrome [68].

A dangerous condition due to extensive serotonin efflux and activation of 5HT-2A receptors in CNS is serotonin syndrome. This syndrome can manifest itself in symptoms ranging from mild (tachycardia, pupils dilated, sweating, agitation), through moderate (tachycardia, elevated temperature and blood pressure, mydriasis, head dyskinesis, hyperreflexia), to severe (severely elevated blood pressure and tachycardia, cardiogenic shock, agitated delirium, muscle rigidity, rhabdomyolysis, acute kidney failure, seizures) that can even lead to death. Serotonin syndrome might come about as a result of using legal or illegal drugs or an interaction between them. This effect is dose-dependent – the higher amount of drug intake, the bigger higher risk of developing serotonin syndrome.

The risk of developing this condition also grows with co-administration of serotonin-releasing drugs. The illicit drugs that can induce serotonin syndrome are mainly stimulants, i.e. amphetamines, cathinones, MDMA, cocaine. Any medication that elevates brain serotonin also may contribute to developing the condition. Briefly presented, examples of such are mainly antidepressants: MAOIs (monoamine oxidase inhibitors) like moclobemide, selegiline, rasagiline; SSRI (serotonin-specific re-uptake inhibitors) like citalopram, escitalopram, fluoxetine, paroxetine; tricyclic antidepressants such as amitriptyline, clomipramine, impramine, doxepin and opipramol; also the opioids – morphine, oxycodone and fentanyl; OTC drugs like pseudoephedrine – and many others. As we can see from the given examples, there is a risk of interaction between mephedrone and many drugs of abuse and medications, and, therefore, the development of life-threatening serotonin syndrome [68]. Data regarding interactions between mephedrone and legal, illegal and prescription drugs are very limited, however, as we know the mechanisms of action and metabolism, we can predict the

effect of combining them. Given the increasing incidence of mental illness and still relatively high level of mephedrone and other cathinones use, there is a need of further investigation of psychiatric drugs and mephedrone`s interactions.

Scientific literature provides no information about food and mephedrone interactions. From the mechanism of action of mephedrone, we can assume that it could interact with products containing tyramine (an indirectly acting sympathomimetic elevating noradrenaline levels). We can assume that consuming tyramine rich food, like blue cheeses, parmesan-like cheeses, pickles, fish sauces, alcohol beverages like wine and beer altogether with mephedrone intake might lead to overstimulation of CNS and hypertension, however this matter need further investigatio [99,100].

Animal behavioural effects

Literature data from preclinical studies provide extensive information about the behavioral effects of mephedrone. This came about as a result of tests involving mice and rats of different breeds and gender, but also non-human primates (rhesus monkeys), as well as flatworms (planarians). They provide data on multiple effects, including locomotor hyperactivity, sensitization, increase in body temperature and abuse liability. These effects are characteristic for psychostimulants. There are some data indicating the development of mephedrone dependence – conditioned place preference, sensitization and tolerance to increased locomotor activity, supporting self-stimulatory behaviours. For a short overview of animal research results see Table 1.

3. SUMMARY AND CONCLUSIONS

Mephedrone is a representative of the synthetic cathinones that before changes in legislation banned their use, were deemed NPS or "legal highs", and were found in mixtures commonly known as "bath salts". Mephedrone use has changed over time. It was first synthesized in 1929, then was forgotten, and has now become one of the most popular recreational drugs, to be used at a low, but relatively constant level. It acts as a psychostimulant, and its effects are among users are comparable to intake of MDMA and cocaine. Its popularity at the turn of the first and second decades of the XX century flourished because of its competitive price, problems in availability and purity of MDMA, its legal status and ease in purchase.

We now know quite a lot about mephedrone`s effects in animals and the neuronal changes it causes. It acts similarly to other psychostimulants, via increasing monoamine concentration in the synaptic cleft. Mephedrone intake exerts a range of behavioural effects. Its administration increases locomotor activity, causes sensitization to hyperlocomotor effects after prolonged, but intermittent administration, and exerts tolerance to hyperlocomotor effect after prolonged use. Moreover it elicit conditioned place preference, substitutes for the discriminative stimulus effects of cocaine and methamphetamine and is willingly self-administered by animals. These effects indicate that it holds an addictive potential. This has been partially confirmed with human-based studies that are limited and mostly comes from questionnaires studies and case reports. Mephedrone users confirm drug craving after expiration of its effects, tolerance to it with prolonged dose (and therefore the need to increase the dose taken) and withdrawal syndrome. Moreover, in literature there are repots of mephedrone-related deaths caused by self-use of a mixture of drugs, but also mephedrone alone. We still do not know what are long lasting effects of mephedrone administration, however, or what neurological impairment it causes, specifically in young adults (as this group use it the most). Well established data regarding its abuse liability and cause of death is, is therefore crucial with regard to understanding this cathinone.

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