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Research article

Monoaminergic toxicity induced by cathinone phthalimide: An *in vitro* study

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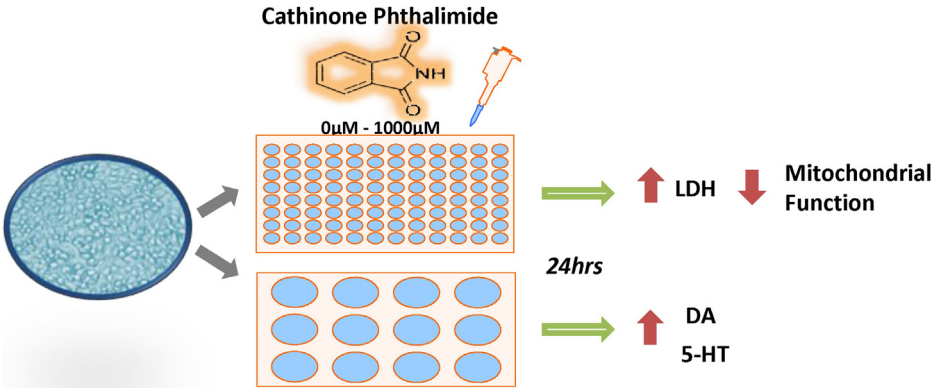
HIGHLIGHTS

- Cathinone phthalimide (CP) increases cell death beginning at 10 μM.
- CP does not affect glutathione, suggesting toxicity may not be from oxidative stress.
- CP alters dopamine and serotonin levels, similar to mephedrone or methylone.

GRAPHICAL ABSTRACT



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ABSTRACT

Bath salts, or synthetic cathinones, have cocaine-like or amphetamine-like properties and induce psychoactive effects *via* their capacity to modulate serotonin (5-HT) and dopamine (DA). Structurally distinct synthetic cathinones are continuously being generated to skirt existing drug laws. One example of these modified compounds is cathinone phthalimide (CP), which has already appeared on the global market. The lack of toxicological studies on the effects of CP on monoaminergic systems led to the development of the present study in order to generate an acute toxicity profile for CP, and to clarify whether it primarily affects both dopamine and serotonin, like the synthetic cathinones mephedrone and methylone, or primarily affects dopamine, like 3, 4-methylenedioxypyrovalerone (MDPV). For the first time, the toxicity profile of CP (10 μM–1000 μM) is reported. In pheochromocytoma cells, exposure to CP induced cell death, and altered mitochondrial function, as well as intracellular DA and 5-HT levels; at the same

Abbreviations: METH, methamphetamine; DA, dopamine; 5-HT, serotonin; MDPV, 3 4-methylenedioxypyrovalerone; CP, cathinone phthalimide; PMS, phenazine methosulfate; LDH, lactate dehydrogenase; GSH, glutathione; XTT, 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide.

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Methylone
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time, reduced glutathione (GSH) levels remained unaffected. This seems to indicate that CP functions like mephedrone or methylone. The role of CP metabolites, the effect of CP induced hyperthermia on neurotoxicity, and its ability to traverse the blood-brain barrier warrant further consideration.

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1. Introduction

Cathinone is a β-keto analogue of amphetamine [22] that is found in the khat plant *Catha edulis*, which is native to East Africa and Southern Arabia [43]. In the United States, it is currently classified as a Schedule I controlled substance under the Controlled Substances Act [1]. Synthetic cathinones are a broad category of drugs commonly present in illicit "bath salt" products, which are typically marketed as inexpensive, and sometimes legal, substitutes for stimulants like methamphetamine (METH) and cocaine [33,46]. Cathinones typically exert their psychostimulant effects *via* interactions with monoamine transporters, either by cocaine-like prevention of reuptake of neurotransmitters like dopamine (DA) and serotonin (5-HT) from the extracellular space back into the cytoplasm of neurons [9], or by amphetamine-like stimulation of efflux of DA and 5-HT [9,36].

Because of the ability to modulate DA and 5-HT, synthetic cathinones have cocaine-like or amphetamine-like properties and therefore induce psychoactive effects [36]. Bath salts, specifically mephedrone, first appeared as a legal product in convenience stores in the early 2000s [6]. While mephedrone was more popular in Europe, 3, 4-methylenedioxypyrovalerone (MDPV) has been the most prevalent bath salt constituent in the United States. In 2011, the DEA temporarily scheduled possession and sale of mephedrone, MDPV, and methylone for 1 year pending additional evaluation [3]. In 2012, the Synthetic Drug Abuse Prevention Act permanently placed several synthetic cathinones into Schedule I of the Controlled Substances Act [35], including mephedrone and MDPV. Methylone was added to the Schedule I list in 2013 [2].

With increasing restrictions on recreational drug use, underground chemists have begun making new synthetic cathinones, in an attempt to subvert current legislation. Synthetic cathinones are being generated that are legal under existing drug laws and intended as alternatives to cocaine, 3,4-methylenedioxymethamphetamine (MDMA), and METH [43]. These compounds are appealing to drug users not only because of their legality, but also their low cost, and because standard drug tests for cocaine, MDMA, and other amphetamines will not trigger a positive result from synthetic cathinones [6,9]. One example of these modified compounds is cathinone phthalimide (CP), also called α-phthalimidopropiophenone or 2-(1-oxo-1-phenylpropan-2-yl)isoindole-1,3-dione, which has already been identified globally as a substance intended for recreational use [43]. For example, Neorganics, an Israel-based internet company, currently offers products containing CP [12]. One large problem with the drugs that produce "legal highs" is that users often assume they are safe because they are not illegal. Further complicating matters is the fact that companies distributing these products frequently change the active constituents of their brand-name products [14,36]. In addition, synthetic cathinones are often found in drugs sold as "Molly," slang for pure MDMA [6]. Some users seek Molly to avoid the additives routinely found in MDMA pills sold as Ecstasy, all the while still exposing themselves to the same risks [18,24]. Therefore, the number of drug users exposed to synthetic cathinones is likely higher than reported, as is the frequency of exposure.

It is also known that synthetic cathinones are a complex group of compounds and their biological activity cannot be inferred from existing data, but rather should be determined on a case-by-case basis [9,17]. Due to the lack of toxicological studies about the effects of CP on the dopaminergic system, the goal of the present study was to generate a basic toxicity profile for CP and to clarify whether it primarily affects dopamine and serotonin, like mephedrone and methylone [10,13,23,46], or primarily affects dopamine, like MDPV [11,26,38].

2. Materials and methods

2.1. Chemicals

Pheochromocytoma cells (PC12) were obtained from American Type Culture Collection (Manassas, VA). Cathinone phthalimide was graciously gifted by Kenner C. Rice from the National Institutes on Drug Abuse (NIDA). Cytotoxicity detection kits (LDH) were purchased from Roche Applied Sciences (Mannheim, Germany). RPMI1640 was purchased from Cellgro (Manassas, VA). Serum was purchased from Atlanta Biologicals (Flowery Branch, GA). Total Glutathione Detection Kit was purchased from Assay Designs (Farmingdale, NY). Pierce® BCA protein assay kit was obtained from Thermo Scientific (Waltham, MA). All remaining media supplements and reagents were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Cell culture

PC12 cells were grown at 37 °C and 5% CO_2 in complete media consisting of RPMI1640 supplemented with 10% horse serum, 5% fetal bovine serum, and 1% penicillin/streptomycin. Upon ~70% confluence, typically 5 days, cells were exposed to various concentrations (10 μM–1000 μM) of CP for 24 h.

2.3. Cell death

Lactate dehydrogenase (LDH) release, from the cytoplasm of damaged cells an indicator of membrane integrity and cell death [27], was measured according to manufacturer specifications. Briefly, after cells were treated with 10 μM–1000 μM CP for 24 h, 100 uL of media was incubated with 100 μL of LDH substrate mixture in a 96 well plate. After 15 min on an incubated shaker, 50 μL of stop solution was added to each well and the absorbance measured at 490 nm with a reference wavelength of 650 nm. Data were analyzed and presented as percent of control cells receiving only media. Three separate experiments were conducted with three replicates of each dose per experiment.

2.4. Mitochondrial function

The mitochondrial function of the PC12 cells was determined using previously described methods [28]. Briefly, cells were treated with 10 μM–1000 μM CP for 24 h. Mitochondrial dehydrogenase-induced cleavage of 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) to generate a water-soluble orange colored formazan derivative was then mea-