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# Cytotoxic, genotoxic, and oxidative stress-related effects of lysergic acid diethylamide (LSD) and phencyclidine (PCP) in the human neuroblastoma SH-SY5Y cell line

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Lysergic acid diethylamide (LSD) is a classic hallucinogen, widely abused for decades, while phencyclidine (PCP) has increased in popularity in recent years, especially among the adolescents. Very little is known about the general toxicity of these compounds, especially about their possible neurotoxic effects at the cell level. The aim of this study was to address these gaps by assessing the toxic effects of 24-hour exposure to LSD and PCP in the concentration range of 0.39–100 µmol/L in the human neuroblastoma SH-SY5Y cell line. After cell viability was established, cells treated with concentrations that reduced their viability up to 30 % were further subjected to the alkaline comet assay and biochemical assays that enable estimation of oxidative stress-related effects. Treatment with LSD at 6.25 µmol/L and with PCP at 3.13 µmol/L resulted with 88.06±2.05 and 84.17±3.19 % of viable cells, respectively, and led to a significant increase in primary DNA damage compared to negative control. LSD also caused a significant increase in malondialdehyde level, reactive oxygen species (ROS) production, and glutathione (GSH) level, PCP significantly increased ROS but lowered GSH compared to control. Treatment with LSD significantly increased the activities of all antioxidant enzymes, while PCP treatment significantly increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) but decreased catalase (CAT) activity compared to control. Our findings suggest that LSD has a greater DNA damaging potential and stronger oxidative activity than PCP in SH-SY5Y cells.

KEY WORDS: antioxidant enzymes; cell viability; DNA damage; GSH; hallucinogenic drugs; psychoactive substances; ROS; toxicity

Lysergic acid diethylamide (LSD) is a classic hallucinogen or psychedelic, and although it does not share the structure and pharmacological properties of dissociative anaesthetic phencyclidine (PCP, aka "angel dust"), both drugs alter perception, mood, and cognition in users (1).

LSD is a serotonin 2A receptor (5-HT2AR) agonist, but the link between receptor activation and cognitive impairment and hallucinations is still poorly understood (2). Apart from recreational use, considerable interest has been seen for its use in clinical research for the treatment of alcohol use disorder, anxiety related to life-threatening conditions, and schizophrenia (3). LSD is mainly taken orally on absorbent (blotter) paper or as tablets (4). A dose of 1–3 µg/kg body weight produces moderate effect within 30–60 min after use. In clinical studies, the usual administered dose is up to 200 µg. The most common presentation of LSD intoxication is psychosis. There is still no evidence of organ impairment or neuropsychological deficits, even at very high doses (2).

PCP acts as *N*-methyl-D-aspartate (NMDA) receptor antagonist. It produces a wide range of dose-dependent effects, from pleasure and excitement at lower doses to anaesthesia at higher doses (4). The analgesic dose is 8–10 mg, while the overdose of ≥25 mg can cause convulsions, respiratory depression, and even death (5). Smoking is its primary administration route, and behavioural effects begin after 5 min at the usual dosage of 1–5 mg (6). In recent years, significant concerns have been raised about its abuse, as it has become popular among young population at dance clubs and parties earning the title of a "club drug". Although knowledge about the use and abuse of hallucinogenic drugs is constantly growing, this research area is still controversial and calls for further investigation.

The aim of our study was to shed more light on possible neurotoxic LSD and PCP effects at the cell level by assessing their cytotoxic and DNA damaging effects, as well as oxidative stress-related phenomena in human neuroblastoma SH-SY5Y cells. This experimental model was chosen as SH-SY5Y cells share many characteristics

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of dopaminergic neurons and are widely used in studying neurotoxic and neuroprotective effects (7). We assumed that such a comprehensive experimental design would bring new and useful evidence for risk assessment.

## MATERIALS AND METHODS

### *Chemicals and reagents*

Analytical standards of the tested drugs LSD and PCP (Lipomed; Arlesheim, Switzerland) were solved in methanol (Merck, Darmstadt, Germany) to obtain the concentration of 1 mg/mL. Other reagents and chemicals used for experiments were bought from Sigma-Aldrich (Steinheim, Germany).

### *Cells*

SH-SY5Y cells (ECACC 94030304, passage numbers 17–23) were obtained from a certified cell-bank, the European Collection of Authenticated Cell Cultures (ECACC), through Sigma-Aldrich. Until treatment, they were cultivated at 37 °C in a 5 % $CO_2$ atmosphere in DMEM F12 medium containing 15 % (v/v) foetal bovine serum, 2 mmol/L glutamine, 1 % (v/v) penicillin/streptomycin, and 1 % (v/v) non-essential amino acids. The medium was replaced every few days.

### *Experimental design*

Three separate experiments for LSD and PCP were conducted by applying the same experimental design.

One day before each experiment, the cells were seeded in 96-well plates at a density of 20,000 cells/well. On the day of the experiment, the cells were exposed to the tested drugs at concentration range of 0.39–100 µmol/L for 24 h according to experimental schedule presented in Table 1. Drug concentrations were selected based on available literature (8–11).

**Table 1** Experimental schedule

| Experimental group | LSD (mg/L) | PCP (mg/L) |
|---|---|---|
| 0.39 µmol/L | 0.13 | 0.09 |
| 0.78 µmol/L | 0.25 | 0.19 |
| 1.56 µmol/L | 0.50 | 0.38 |
| 3.13 µmol/L | 1.01 | 0.76 |
| 6.25 µmol/L | 2.02 | 1.52 |
| 12.50 µmol/L | 4.04 | 3.04 |
| 25 µmol/L | 8.09 | 6.08 |
| 50 µmol/L | 16.17 | 12.17 |
| 100 µmol/L | 32.34 | 24.34 |
| Control | untreated cells | |

During treatment, cell cultures were grown at 37 °C in a 5 % $CO_2$ atmosphere. Untreated cells were used as negative control.

After the treatment, the cells were washed with phosphate-buffered saline (PBS, pH 7.4) and analysed with the MTS assay, alkaline comet assay, and biochemical assays for oxidative stress parameters.

### *Measurement of cell viability – MTS assay*

The cytotoxic properties of the tested drugs were studied by measuring mitochondrial succinate dehydrogenase activity with the MTS detection reagent assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA) according to the test protocol reported by Zandona et al. (12). Briefly, the treated cells were rinsed with PBS buffer. Then, 100 µL of DMEM F12 and 20 µL of MTS reagent were pipetted in each well, and cultures incubated for up to 3 h. After that, absorbance was read on a plate reader (Infinite M200PRO, Tecan Austria GmbH, Salzburg, Austria) at 492 nm. Data were pooled from three experiments with duplicate or triplicate evaluations. Results are reported as percentages (mean ± standard deviation) of viable cells with respect to control.

Given that for reliable genotoxicity assessment viability has to be over 70 % (13), for further LSD toxicity assessment we selected the concentration of 6.25 µmol/L, which resulted with the highest cytotoxicity in this experiment. The effects of PCP were further studied at 3.13 µmol/L, as this concentration produces comparable cytotoxicity with LSD, and falls among the values relevant for human exposure *in vivo* (10, 11).

### *Alkaline comet assay*

DNA damaging effects were studied using the alkaline comet assay, whose effectiveness in monitoring DNA damage in neural cells has been well established (14). The extent of DNA damage in single cells was assessed with the standard alkaline comet assay (15) with minor adjustments. Agarose microgels were prepared on fully frosted microscope slides (Surgipath®, Cambridgeshire, UK). For each experimental point, duplicate slides were prepared. The first layer of gel consisted of 0.6 % normal melting point (MP) agarose. It was then covered with (i) a mixture of 0.5 % low MP agarose and cell samples (V=15 µL of suspension containing $10^5$ cells per slide) and (ii) the top layer of 0.5 % low MP agarose.

To prepare positive control slides, we exposed microgels with untreated cells to 50 µmol/L hydrogen peroxide on ice for 10 min. Hydrogen peroxide was selected, as it induces extensive DNA damage, detectable by the comet assay. Generally, such use of an established genotoxic substance as positive control is recommended to achieve positive response comparable to that of tested substances (16).

Prepared slides were all processed further in the same way. Following polymerisation, microgels were immersed

overnight in a lysis buffer (pH=10) composed of 100 mmol/L $Na_2EDTA$, 2.5 mol/L NaCl (Kemika, Zagreb, Croatia), 10 mmol/L Tris-HCl, 1 % *N*-lauroylsarcosine sodium salt, 1 % Triton™ X-100, and 10 % dimethyl sulphoxide (Kemika). Gels were then placed for 20 min in a denaturation buffer (pH >13) composed of 300 mmol/L NaOH (Kemika) and 1 mmol/L $Na_2EDTA$. The same buffer was used for electrophoresis, which was run at 25 V, 300 mA, and 4 °C for 20 min. Slides were then rinsed three times with 0.4 mol/L Tris-HCl buffer (pH=7.5). Microgels stained with ethidium bromide (20 µg/mL) were observed under a fluorescence microscope (Olympus BX50, Tokyo, Japan) at 200× magnification. Fifty randomly selected comets were scored for each of the two microgels using the Comet Assay IV™ software (Instem-Perceptive Instruments Ltd., Suffolk, Halstead, UK). As the experiment was carried out in triplicate, altogether 300 comets for each sample were measured. The degree of DNA damage was defined using two comet descriptors: tail DNA% and tail length (presented in micrometres).

*Malondialdehyde determination*

Malondialdehyde (MDA) is a reactive end-product of polyunsaturated fatty acid peroxidation. Its levels were determined using the method described by Khoschsorur et al. (17). Treated cells were washed twice in the Krebs-Heneseleit buffer, centrifuged at 248 *g* for 5 min, and lysed by sonication on ice. Cell lysates were then resuspended in 250 mL of the Krebs-Heneseleit buffer. The reaction mixture consisted of 25 µL of cell lysate (or standard, i.e. 2.5 µmol/L 1,1,3,3-tetraethoxy propane), 225 µL of ultrapure water (18 MΩ, Milli-Q Gradient water system, Thermo Scientific Smart2pure 3 UV/UF, Thermo Electron LED GmbH, Langenselbold, Germany), 375 µL of phosphoric acid (0.44 mol/L), and 125 µL of thiobarbituric acid (TBA, 42 mmol/L, Sigma Chemicals, St. Louis, MO, USA) and kept in a hot water bath for 30 min.

The analysis was performed using a HPLC instrument with UV detector (Shimadzu Corporation, Kyoto, Japan). The guard column and analytical column were C-18 reverse-phase (LiChrospher, Merck) with 5 µm particles (4.0x4.0 mm and 4.0x125.0 mm, respectively).

The mobile phase was 50 mmol/L $KH_2PO_4$ (Merck) and methanol (60:40, v/v, pH 6.8, Sigma Chemicals). The injection volume was 50 µL, flow-rate 1 mL/min, while absorbance at fluorescence detector was set to 527 (λex) and 550 nm (λem). The retention time of MDA was about 2.5 min. MDA concentration was calculated from the calibration curve using the instrument's software (Shimadzu LCsolution) and expressed as nmol/mL.

*Reactive oxygen species detection*

The content of intercellular reactive oxygen species (ROS) was determined using a fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). After this compound enters a cell, it is first converted into a non-fluorescent product that, in the presence of peroxyl, hydroxyl, and other ROS, is further oxidised to the fluorescent product 2',7'-dichlorodihydrofluorescein (DCF) (18).

Cells were cultivated in the same medium and manner as for other assays, at 37 °C in a $CO_2$ incubator. The measurements were done in triplicate in dark-sided 96-well microplates. 100 µL of cell suspension containing $10^4$ cells was pipetted into each well. To assess the amount of intercellular ROS, the culture medium was discarded, and the cells rinsed with 100 µL of PBS and incubated with 200 µL of 20 µmol/L DCFH-DA at 37 °C for 30 min. The dye was then discarded, and the cells rinsed again with 100 µL of PBS and treated with the same concentrations of drugs as in other assays. Fluorescence was recorded on a multilabel plate reader (Victor3™, Perkin Elmer, Waltham, MA, USA) at the excitation wavelength of 485 nm and emission wavelength of 535 nm. The obtained fluorescence arbitrary units were converted into percentages of control values.

*Measurement of glutathione levels*

Intracellular glutathione (GSH) was measured with the fluorimetric assay based on the use of fluorogenic monochlorobimane probe (mBCl). Reaction with GSH results in the formation of a fluorescent product in an amount proportional to the content of GSH (19).

Cell cultures in dark-sided 96-well plates were prepared in the same medium and conditions as for other assays. Each well contained 100 µL of cell suspension with $10^4$ cells. Cells were treated with the same concentrations of drugs as in other assays, followed by a 4-hour incubation at 37 °C in a $CO_2$ incubator. After rinsing with PBS, cells were incubated with 200 µL of 20 µmol/L mBCl in PBS at 37 °C for 30 min. The concentration of GSH in cell samples was measured with a Victor3™ multilabel plate reader (Perkin Elmer) at the excitation wavelength of 355 nm and emission wavelength of 460 nm. The obtained fluorescence arbitrary units were then converted into percentages of control values. The measurements were done in triplicate.

*Antioxidant enzyme activity assays*

Antioxidant enzyme activities were measured in triplicate in the cells seeded in 96-well plates and recorded on a plate reader (Tecan Infinite M200PRO, Tecan Austria GmbH). Treated cells were harvested with a rubber policeman and then centrifuged to remove the lysate buffer at 2,000 *g* and 4 °C for 10 min.

Glutathione peroxidase (GPx) activity was measured with the Assay Kit No.703102 (Cayman Chemical Company, Ann Arbor, MI, USA) according to producer's instructions (20). After a sequence of reactions with this kit, the decline in absorbance of NADPH (as it oxidises into $NADPH^+$) at 340 nm is directly proportional to GPx activity

in the sample. One unit of activity corresponds to the amount of GPx needed to catalyse oxidation of 1 nmol of NADPH per minute and per milligram of protein.

Superoxide dismutase (SOD) activity was measured with the Assay Kit No. 706002 (Cayman Chemical Company) according to producer's instructions (21). The assay uses a tetrazolium salt to detect superoxide radicals produced by xanthine oxidase and hypoxanthine. One unit of activity corresponds to the amount of SOD needed to produce 50 % dismutation of the superoxide radical. SOD activity is expressed as U/mg of protein.

Catalase (CAT) activity was measured with the Assay Kit No. 707002 (Cayman Chemical Company) according to producer's instructions (22). The method relies on enzyme reaction with methanol in the presence of $H_2O_2$ at an optimal concentration, which yields formaldehyde. Its amount was measured colourimetrically at 540 nm with 4-amino-3-hydrazino-5-mercapto-1,2,3-triazole (Purpald®, Sigma-Aldrich) used as the chromogen. One unit of activity corresponds to 1 nmol of formaldehyde per minute and per milligram of protein.

Protein content was determined with the QuantiPro™ BCA Assay Kit (Sigma-Aldrich) according to producer's instructions. The assay relies on the production of a $Cu^{2+}$-protein complex under alkaline conditions, accompanied by a reduction of the $Cu^{2+}$ to $Cu^{+}$. The extent of this reduction is proportional to protein concentration.

*Statistical analysis*

Besides descriptive statistics for each experiment, cell viability data, whose means more accurately represent the centre of distribution, were analysed with parametric one-way analysis of variance (ANOVA) and Dunnett's test using the Prism 8 software (GraphPad Software, San Diego, CA, USA).

Data with non-parametric distribution (whose medians more accurately represented the centre of distribution), such as the comet assay and biochemical assay were analysed with the Mann-Whitney *U*-test on Statistica version 14. (TIBCO Software, Inc., Palo Alto, CA, USA).

Statistically significance was set at $p<0.05$.

## RESULTS

*Cell viability*

Table 2 shows SH-SY5Y cell viability after 24 h of exposure to LSD and PCP in the concentration range of 0.39–100 µmol/L. Compared to control, LSD significantly decreased cell viability in the concentration range from 0.78 to 6.25 µmol/L, and PCP in the concentration range from 1.56 to 100 µmol/L.

*Genotoxicity*

Results of the alkaline comet assay (Table 3) demonstrate statistically significant increase in tail DNA% and tail length after LSD treatment and significant increase tail length after PCP treatment compared to negative control.

Figure 1 also shows comet frequency distribution in treated and control cells by tail DNA% and length divided into four quartiles. With LSD the number of comets in the <25$^{th}$ percentile significantly decreased for both tail DNA% and length, while the proportion of those exceeding the 75$^{th}$ percentile significantly increased compared to control. With PCP, we observed no significant differences in tail DNA% pattern, but the number of comets in the 50$^{th}$–75$^{th}$ and >75$^{th}$ length percentile increased significantly.

*Oxidative stress-related effects*

Oxidative stress-related effects determined in SH-SY5Y cells after treatments with 6.25 µmol/L (2.02 mg/L) LSD, 3.13 µmol/L (0.76 mg/L) PCP and negative control are shown in Figures 2–7.

**Table 2** Viability of SH-SY5Y cells exposed to LSD and PCP in concentrations of 0.39–100 µmol/L for 24 h determined with the MTS assay

| Experimental group | Viable cells (mean % ±SD) | |
|---|---|---|
| | LSD | PCP |
| Control | 99.67±3.74 | 99.20±4.42 |
| 0.39 µmol/L | 97.77±7.94 | 92.65±6.25 |
| 0.78 µmol/L | 93.68±8.35* | 90.05±7.07 |
| 1.56 µmol/L | 90.26±7.52* | 86.54±11.08* |
| 3.13 µmol/L | 90.67±5.95* | 84.17±9.03* |
| 6.25 µmol/L | 88.06±7.44* | 78.10±5.49* |
| 12.5 µmol/L | 94.18±8.18 | 76.28±10.10* |
| 25 µmol/L | 100.17±7.29 | 75.57±9.15* |
| 50 µmol/L | 101.82±6.77 | 71.02±13.29* |
| 100 µmol/L | 98.09±7.63 | 72.80±8.85* |

* $P<0.05$ vs control (one-way ANOVA followed by Dunnett's test)

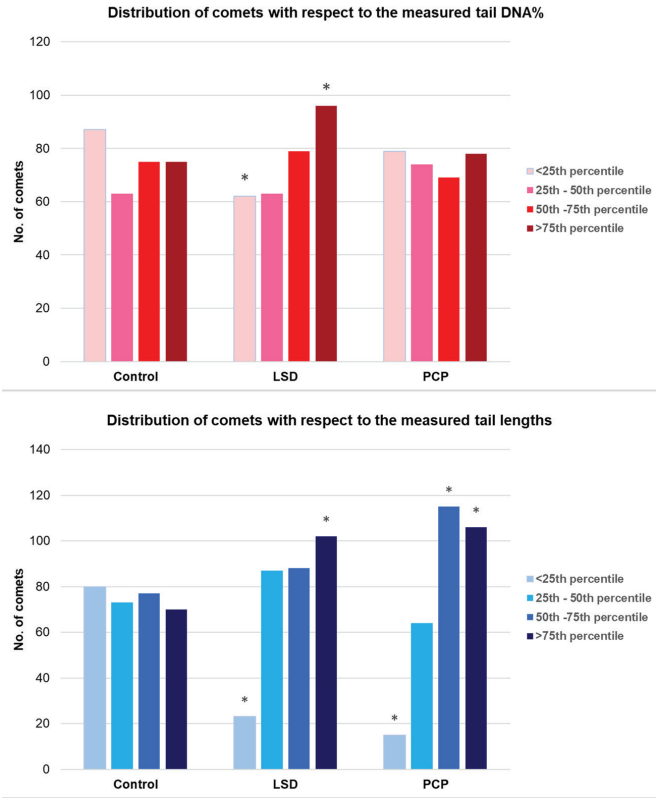


**Figure 1** Frequency distribution of DNA damage in SH-SY5Y cells after treatment with LSD (6.25 µmol/L) and PCP (3.13 µmol/L) compared to control (untreated cells). *p<0.05 vs control (Pearson's $\chi^2$ test)

Figure 2 shows that 24 h treatment with LSD significantly increased MDA level compared to control, while PCP had the opposite effect.

Figure 3 shows a significant increase in DCFA fluorescence with LSD treatment and, therefore, a significantly higher ROS production compared to control.

Figure 4 shows that LSD significantly increased GSH level compared to control, while PCP had the opposite effect.

Both LSD and PCP significantly increased GPx (Figure 5) and SOD activities compared to control (Figure 6). LSD also significantly increased CAT activity, while PCP had the opposite effect (Figure 7).

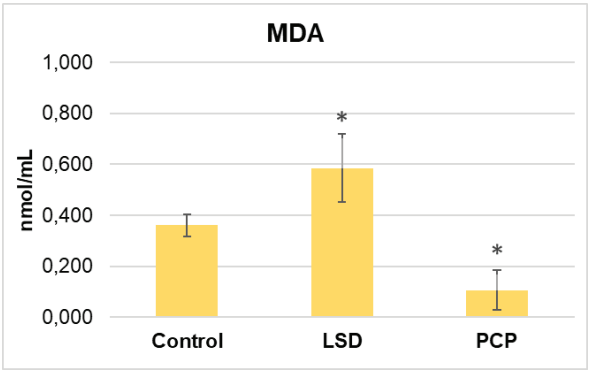


**Figure 2** Concentration of malondialdehyde (MDA) measured in SH-SY5Y cells after treatment with LSD (6.25 µmol/L) and PCP (3.13 µmol/L) compared to control. *p<0.05 vs control (Mann-Whitney *U* test)

## DISCUSSION

Our preliminary *in vitro* study has provided the first evidence of LSD and PCP cytotoxicity, DNA damage, and oxidative stress in SH-SY5Y cells, and we hope it will further the understanding of their toxicity profiles and help to design future experiments.

LSD did not significantly reduce cell viability over a wide range of tested concentrations, but at its most cytotoxic concentration of 6.25 µmol/L this compound produced significant genotoxic effects, oxidative stress, and oxidative-antioxidant imbalance. PCP, in turn, showed a slightly higher and concentration-dependent cytotoxicity, even though it was tested at lower micromolar concentrations. Interestingly though, its higher cytotoxicity was not accompanied by proportionate DNA damage or oxidative stress-related effects, which suggests that the tested compounds do not share the same mechanisms of action.

In our experiment, none of the applied concentrations killed more than 30 % of the cells. However, this does not mean that they did not start a cascade of reactions that could trigger other mechanisms with long-term effects. Namely, drugs can have various adverse effects on the redox metabolism in mitochondria, phospholipid metabolism, and proteolytic pathways, all of which result in ROS production. While low ROS levels are needed for normal cell signalling, higher concentrations damage DNA, lipids, and proteins (23), which happened in our study as well. To protect

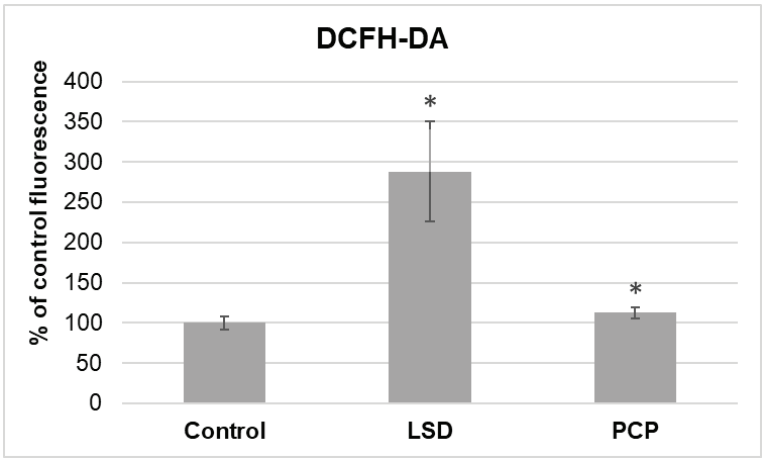


**Figure 3** ROS production in SH-SY5Y cells measured with the DCFH-DA assay after treatment with LSD (6.25 μmol/L) and PCP (3.13 μmol/L) compared to control. *p<0.05 vs control (Mann-Whitney *U* test); DCFH-DA – 2',7'-dichlorodihydrofluorescein diacetate

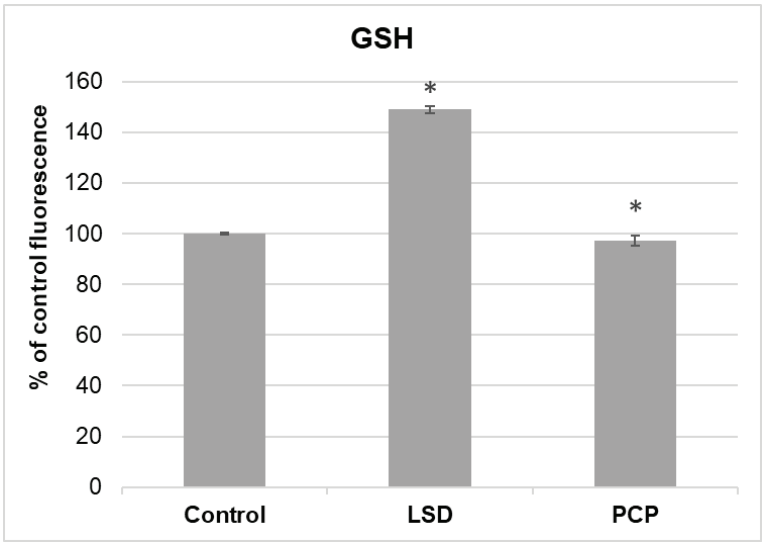


**Figure 4** Glutathione (GSH) level measured in SH-SY5Y cells after treatment with LSD (6.25 μmol/L) and PCP (3.13 μmol/L) compared to control. *p<0.05 vs control (Mann-Whitney *U* test)

themselves, cells respond by increasing antioxidant enzyme activity, such as that of GPx, SOD, and CAT, to restore redox balance. An important role in maintaining homeostasis is also played by GSH, which is essential for normal cell growth (24). This struggle to obtain homeostasis is evident in the increased levels of MDA, ROS, GSH, and antioxidant enzyme activities after treatment with LSD in our study. MDA increase was obviously the result of ROS attack on polyunsaturated fatty acids in lipids (25), and we know that LSD and PCP affect mitochondria, since mitochondrial succinate dehydrogenase activity was measured in our MTS assays and resulted in increased ROS levels due to a disturbance in the oxidative phosphorylation chain, as reported by other studies in other cells (26, 27) with similar psychoactive substances (28). Other investigators (29) also showed that prolonged exposure to 3 μmol/L PCP resulted in cell apoptosis and up-regulation of the *N*-methyl-D-aspartate (NMDA) receptor. This means that, if we had prolonged our exposure, all the effects observed in the first 24 h would probably have led to apoptosis.

Until now, the DNA damaging potential of both tested compounds has been poorly investigated, which makes our findings a very important contribution to the existing knowledge on this subject. From the results obtained by the alkaline comet assay we can conclude that both LSD and PCP are able to produce primary DNA damage at the tested concentrations, which is evident from the values of two comet descriptors, tail DNA% and tail length. Tail DNA% is generally considered the most useful descriptor of damage detected by the alkaline comet assay. It indicates how much of the broken DNA ended in the comet tail and correlates with the incidence of DNA breaks. Comet tail length is a particularly useful descriptor of low levels of damage (30). However, as it is determined by the length of DNA loops, when the comet tail is formed, its length quickly gains its maximum (30, 31). This is why a combination of these two comet descriptors provide a more accurate measure of total primary DNA damage.

Speaking of the extent and nature of DNA damage produced by LSD and PCP, we cannot provide definite

**Table 3** Primary DNA damage measured in SH-SY5Y cells using the alkaline comet assay after treatment with 6.25 µmol/L (2.02 mg/L) LSD, 3.13 µmol/L (0.76 mg/L) PCP, and in respective negative and positive controls

| Sample | Negative control (untreated cells) | | LSD | | PCP | | Positive control ($H_2O_2$) | |
|---|---|---|---|---|---|---|---|---|
| **Descriptor** | **Tail DNA%** | **Tail length (µm)** | **Tail DNA%** | **Tail length (µm)** | **Tail DNA%** | **Tail length (µm)** | **Tail DNA%** | **Tail length (µm)** |
| **Mean** | 1.57 | 13.55 | 3.14* | 15.46* | 2.30 | 15.65* | 8.33* | 15.84* |
| **SE** | 0.14 | 0.26 | 0.33 | 0.32 | 0.25 | 0.27 | 0.66 | 0.39 |
| **SD** | 2.43 | 4.53 | 5.68 | 5.51 | 4.27 | 4.60 | 11.51 | 6.84 |
| **Median** | 0.22 | 12.50 | 0.62 | 13.75 | 0.21 | 14.58 | 2.87 | 13.75 |
| **Minimum** | 0 | 6.25 | 0 | 7.50 | 0 | 7.92 | 0 | 5.00 |
| **Maximum** | 11.97 | 28.33 | 45.77 | 54.17 | 29.28 | 37.08 | 63.81 | 45.83 |

The results of descriptive statistics relied on three hundred independent comet measurements per experimental group. *significantly higher compared to negative control (P<0.05; Mann-Whitney *U* test)

answers. The main reason is the specificity of the comet assay, whose alkaline version identifies a wide array of lesions: strand breaks (single and double), labile sites in DNA that can be converted into breaks by treatment with alkali, single-strand breaks owed to incomplete excision repair, and DNA-DNA or DNA-protein crosslinks (13, 30–32). Furthermore, a portion of DNA damage is owed to the DNA repair processes, which introduces additional lesions that are detected by the comet assay. This could be considered a limitation of the applied experimental design. However, it is common to use the alkaline method for preliminary assessments, while a more specific enzyme-modified comet assay is usually performed later on.

The next question we have to ask ourselves is why did PCP cause greater tail length, even though it was applied at lower micromolar concentrations than LSD? To answer this question, one should look at the molecular structures of both compounds and/or their primary metabolites. It is known that this compound metabolises into the iminium ion (33), which is a potent bioactive toxic agent involved in DNA damage, oxidative stress, and various physiological responses (34). It has not been documented yet how the iminium form of PCP acts at the genome level, but this reactive species possibly intercalates in the DNA molecule with a similar mechanism of action as has been evidenced for the iminium form of the alkaloid sanguinarine (35). LSD binding to DNA, in contrast, was confirmed more than five decades ago (36). A little later, Wagner (37) suggested that LSD interacted with and caused conformational changes in DNA by intercalation. Many investigators reported chromosomal damage inflicted by LSD (38–40). Since at the time when these studies were conducted sensitive methods such as the comet assay had not yet been developed, it was not possible to clearly associate the level of chromosomal damage to specific events at the DNA molecule level.

Based on available literature data and our own findings, we can propose that both LSD- and PCP-induced DNA damage is related to intercalation of these compounds into

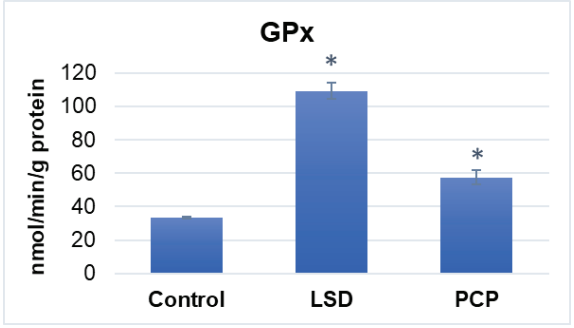


**Figure 5** Glutathione peroxidase (GPx) activity measured in SH-SY5Y cells after treatment with LSD (6.25 µmol/L) and PCP (3.13 µmol/L) compared to control. *p<0.05 vs control (Mann-Whitney *U* test)

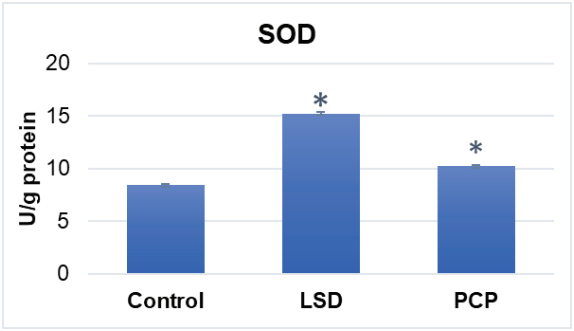


**Figure 6** Superoxide dismutase (SOD) activity measured in SH-SY5Y cells after treatment with LSD (6.25 µmol/L) and PCP (3.13 µmol/L) compared to control. *p<0.05 vs control (Mann-Whitney *U* test)

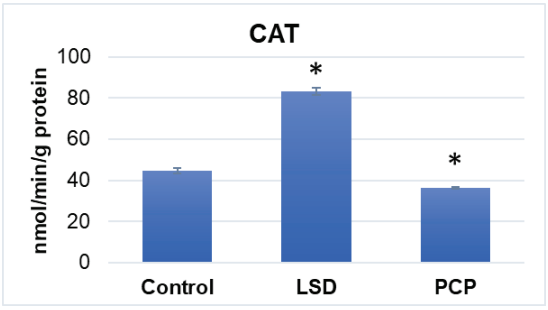


**Figure 7** Catalase (CAT) activity measured in SH-SY5Y cells after treatment with LSD (6.25 µmol/L) and PCP (3.13 µmol/L) compared to control. *p<0.05 vs control (Mann-Whitney *U* test)

DNA. As known, intercalation of a compound into the DNA structure results in various conformational problems that can affect both the denaturation and electrophoresis steps of the comet assay. Intercalation, in addition, should activate various complex DNA repair mechanisms, which may cause additional damage detectable by the alkaline comet assay. As the treatment lasted 24 h, the observed tail DNA% and lengths reflect an equilibrium between the onset and repair of DNA damage over that time.

What remains unanswered is the fate of the observed DNA damage, its stability, and persistence. Among the primary DNA lesions there are those that are completely repaired (such as alkali-labile sites and single strand breaks) and those that require assistance of more complex repair mechanisms or cannot be fully repaired. The latter are of special concern, as they can result with genome instability responsible for long-term detrimental effects. However, their extent and outcomes could not be determined with the present experimental design, and require more sophisticated molecular biology and cytogenetic methods.

The applied experimental design is limited to the conditions and cell type used. Since the SH-SY5Y cell line is derived from the neuroblastoma tumour, the changes observed with this model may differ from those that would occur in healthy neuronal cells, especially in *in vivo* conditions. Even so, the increased oxidative stress and DNA damage we documented in our study calls for concern, as similar effects in normal neuronal tissues cannot be completely dismissed.

Before we can draw any conclusion relevant for a real *in vivo* situation, we should also take into account all the limitations of the *in vitro* model as such. However, even with these limitations in mind, it is plain to see that LSD and PCP can impair the viability and genome stability of neurons. As these terminally differentiated cells do not divide and generally employ much slower DNA repair mechanisms, this could eventually cause their steady loss and have neurotoxic consequences. Since this study was limited to single LSD and PCP doses, potential detrimental effects of these compounds should be further explored on a broader range of doses and on other experimental models.

## CONCLUSION

With our complex experimental design involving five essays we were able to cover all relevant processes triggered by LSD and PCP activity in SH-SY5Y cells and show their cytotoxic potential. Further studies should explore whether these effects are observed in other types of neuronal cells and elucidate which specific mechanisms are responsible for them.

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**Citotoksično i genotoksično djelovanje i oksidacijski stres uzrokovan dietilamid lizerginskom kiselinom i fenciklidinom u staničnoj liniji humanih neuroblastoma SH-SY5Y**

Dietilamid lizerginske kiseline (LSD) klasični je halucinogen koji se desetljećima naširoko zlorabio, dok je posljednjih godina porasla popularnost fenciklidina (PCP), osobito među adolescentima. Do sada se vrlo malo zna o općoj toksičnosti ovih spojeva, posebice o njihovim mogućim neurotoksičnim učincima na staničnoj razini. Ovo istraživanje procijenilo je toksične učinke LSD-a i PCP-a na staničnu liniju humanoga neuroblastoma SH-SY5Y, koja je bila izložena ispitivanim spojevima u rasponu koncentracija 0,39–100 µmol/L tijekom 24 sata. Stanice tretirane odabranim koncentracijama koje su smanjile vijabilnost stanica do 30 % potom su podvrgnute alkalnom komet-testu i biokemijskim testovima koji omogućuju procjenu oksidacijskoga stresa. Tretman s LSD-om od 6,25 µmol/L i PCP-om s 3,13 µmol/L rezultirao je s 88,06±2,05 % odnosno 84,17±3,19 % vijabilnih stanica. Ove koncentracije omogućile su ispitivanje genotoksičnosti, što je rezultiralo značajnim povećanjem primarnog oštećenja DNA nakon tretmana LSD-om i PCP-om u odnosu na negativnu kontrolu. LSD je izazvao značajno povećanje razine malondialdehida u usporedbi s kontrolom, za razliku od PCP-a. Dok je LSD inducirao značajno povećanje proizvodnje reaktivnih kisikovih vrsta (ROS) i razine glutationa (GSH), tretman PCP-om uzrokovao je značajno povećanje proizvodnje ROS-a, ali smanjenje razine GSH-a u usporedbi s kontrolom. Tretman stanica LSD-om značajno je povećao aktivnosti svih antioksidacijskih enzima u usporedbi s kontrolom. Tretman PCP-om značajno je povećao aktivnosti glutation peroksidaze (GPx) i superoksid dismutaze (SOD), ali je aktivnost katalaze (CAT) bila značajno niža nego u odgovarajućoj kontroli. Zaključno, LSD je imao veći potencijal oštećenja DNA i pokazao je jaču oksidacijsku aktivnost od PCP-a u stanicama SH-SY5Y.

KLJUČNE RIJEČI: antioksidacijski enzimi; halucinogene droge; LSD; oštećenje DNA; PCP; psihoaktivne tvari; toksičnost; vijabilnost stanica