Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Analysis of serum levels of organochlorine pesticides and related factors in Parkinson's disease

Shaoqing Xu
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital
Xiaodong Yang
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital
Yiwei Qian
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital
Dayong Wan
The First People's Hospital of Tonglu
Fenghua Sun
The First People's Hospital of Tonglu
Qian Luo
Shanghai Jiao Tong University
Yanyan Song
Shanghai Jiao Tong University
Qin Xiao (xq10537@rjh.com.cn)
Ruijin Hospital, Shanghai jiaotong University School of medicine https://orcid.org/0000-0001-5554-1443

Letter

Keywords: Parkinson's disease, organochlorine pesticides, gene and pesticide interaction

DOI: https://doi.org/10.21203/rs.3.rs-64368/v3

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Background: There is evidence that genetic and environmental factors contribute to the onset and progression of Parkinson's disease (PD). Pesticides are a class of environmental toxins that are linked to increased risk of PD. However, few studies have investigated the interaction between specific pesticides and genetic variants related to PD in the Chinese population.

Methods: In this cross-sectional study, 19 serum levels of pesticides were measured. In addition, we also analyzed the interaction between specific pesticides and candidate genetic variants for PD. Finally, we investigated the mechanistic basis for the association between pesticides and increased risk of PD.

Results: Serum levels of organochlorine pesticides including α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, δ-HCH, propanil, heptachlor, dieldrin, hexachlorobenzene, p,p'-dichlorodiphenyltrichloroethane (p,p'-DDE) and o,p'-dichloro-diphenyl-trichloroethane (o,p'-DDT) were higher in PD patients than in controls. α-HCH and propanil levels were associated with increased PD risk. Serum levels of dieldrin were associated with Hamilton Depression Scale and Montreal Cognitive Assessment scores in PD patients. Interactions between high pesticide levels and polymorphisms in rs11931074 and rs16940758 (α-HCH or β-HCH interacted with TT genotype in rs11931074 and δ-HCH interacted with TT genotype in rs16940758) were associated with the risk of PD. In cell model, α-HCH and propanil increased the level of reactive oxygen species and decreased the mitochondrial membrane potential. Propanil but not α-HCH induced the aggregation of α-synuclein.

Conclusions: Elevated serum levels of α-HCH and propanil are associated with increased risk of PD. Serum levels of dieldrin were associated with depression and cognitive function in PD patients. The interaction between genetic variants and pesticides also increased the risk of PD. Effects of genetic variants and pesticides on the risk of PD should be studied in more detail with a larger sample size to further understand the mechanisms involved.

Background

There is evidence that genetic and environmental factors contribute to the onset and progression of Parkinson's disease (PD). Pesticides are widespread environmental toxicants that are classified into organochlorine pesticides (OCPs), organophosphorus pesticides (OPs) and inorganic pesticides based on their chemical structure. One epidemiological study using 319 cases and 296 controls demonstrated that exposure to pesticides was associated with increased risk of PD [1], as well as faster progression of motor and cognitive scores in PD patients. Fleming et al. also found that pesticide dieldrin was more easily detected in the postmortem brain of PD [2]. In addition, studies reported that genetic variation, for example, rs3775423 polymorphism in α-synuclein (*SNCA*) gene and rs1045642 in ATP-binding cassette, sub-family B, member 1 (*ABCB1*) gene interacted with OCPs exposure to increase PD risk. However, there have been not reports about the association between specific types of pesticides and PD in China, although it was one of the first countries to adopt the use of pesticides.

In this study, we attempt to evaluate the serum level of 19 pesticides (including 16 OCPs and 3 OPs) in relation to PD risk and the effect of gene-environment interaction between selected gene polymorphisms and pesticide levels on patients with PD in Chinese populations. The association between pesticides and clinical features of PD was also investigated. Finally, we investigated the potential mechanisms of OCPs in neurons to determine how these agents may contribute to the development of PD.

Methods

A total of 90 idiopathic PD patients (including 44 from the urban area in Shanghai City and 46 from the rural area of Tonglu County in Hangzhou City) and their healthy spouses from the same household were enrolled in this study. Demographic and clinical characteristics of the study population are shown in Table e-1.

All the examined pesticides were present in any of the samples (Table e-2). Of all, α-HCH (α-hexachlorocyclohexane, α-HCH), β-HCH, aldrin, and p,p'-DDE (p,p'-dichlorodiphenyltrichloroethane, p,p'-DDE) were more easily detected in PD patients compared to controls. Serum concentration of pesticides are shown in Table 1. In the overall population, mean levels of α-HCH, β-HCH, γ-HCH, δ-HCH, propanil, heptachlor, dieldrin, hexachlorobenzene, p,p'-DDE and o,p'-DDT (o,p'-dichloro-diphenyl-trichloroethane, o,p'-DDT) were significantly higher in PD group than in control group (also shown in Fig. e-1).

Due to shrew distribution (not normally distributed), the levels of pesticides were divided into tertiles as indicated by the distribution of concentrations of pesticides in controls. After adjusting for sex, age and region, α-HCH, β-HCH, δ-HCH, propanil, heptachlor, dieldrin, p,p'-DDE and o,p'-DDT were associated with an increased risk for PD (Table e-3). After adjusting for sex, age, region and pesticides levels, α-HCH and propanil concentrations were positively associated with risk of PD (Table e-3). Two studies in US and one study in Faroe Islands found that elevated levels of β-HCH were associated with PD after adjusting for sex and age or smoking [3-5]. However, when pesticides were added as a confounder, only α-HCH and propanil were associated with PD in our study. Others have reported that only dieldrin [6] or β-HCH and dieldrin were associated with PD when pesticides were included as confounders in the analysis [7]. There are several possible reasons for the discrepancies in the results, such as different types and amount of pesticides that were used, racial and ethnic differences, and the fact that healthy spouses were recruited as controls in our study. Studies with larger sample sizes and across multiple centers are needed to explore the relationship between OCPs concentrations and PD risk in greater detail.

Table 1. Serum levels of pesticides in PD patients and controls (ng/g lipid)

Pesticides	Total (n=180)			Urban area (n=88)			Rural area (n=92)		
	PD (n=90)	Con (n=90)	P value	PD (n=44)	Con (n=44)	P value	PD (n=46)	Con (n=46)	P
α-HCH	80.19±51.54	1.79±0.29	<0.01**	88.95±82.04	1.58±0.31	0.1	71.80±64.23	1.98±0.49	<
β-HCH	1126.40±255.69	349.88±90.63	0.01*	642.12±347.95	342.4±124.80	0.4	1589.62±364.09	357.02±132.45	<
γ-HCH	583.30±189.10	39.62±17.16	0.03*	8.02±1.92	8.64±2.07	0.5	1133.57±353.00	69.24±33.11	
δ-HCH	12.09±2.12	5.82±1.33	<0.01**	17.36±3.79	8.36±2.50	0.1	7.04±1.78	3.40±0.95	<
Propanil	29.48±4.12	18.06±3.02	<0.01**	22.61±6.58	14.07±3.99	0.01*	36.04±4.91	21.88±4.48	<
Vinclozolin	21.76±9.10	9.49±0.98	0.3	16.29±5.37	9.53±1.26	0.7	27.00±17.11	9.46±1.5	
Heptachlor	45.00±11.81	1.50±0.15	<0.01**	26.94±17.18	1.52±0.20	0.6	62.26±16.02	1.48±0.21	<
Aldrin	33.90±4.35	31.73±4.62	0.1	30.52±6.16	35.75±7.57	0.7	37.14±6.18	27.89±5.42	
Endosulfan	26.89±7.20	15.27±2.92	0.1	31.44±13.60	15.34±3.95	0.5	22.54±5.54	15.19±4.32	
Dieldrin	14.18±1.18	10.29±1.11	<0.01**	14.44±1.57	7.13±1.16	<0.01**	13.93±1.75	13.31±1.77	
Hexachlorobenzene	375.39±95.87	65.46±12.79	0.02*	22.88±5.74	22.93±5.53	0.8	712.56±174.25	106.14±23.01	<
Quintozene	661.71±151.37	349.35±95.94	0.1	499.46±238.93	380.74±147.72	0.8	816.91±188.11	319.31±125.02	<
p,p'-DDE	149.52±29.28	53.30±11.68	<0.01**	21.03±4.94	9.30±1.282	0.1	272.42±51.05	95.39±21.12	<
p,p'-DDD	14.89±2.28	11.99±1.82	0.3	15.55±3.72	10.25±1.93	0.5	14.26±2.74	13.66±3.05	
o,p'-DDT	10.35±1.15	6.85±0.91	<0.01**	10.70±1.87	5.60±0.78	0.04*	10.01±1.38	8.05±1.61	
p,p'-DDT	42.94±7.20	36.29±5.35	0.4	42.84±12.22	38.07±7.40	1.0	43.04±8.03	34.60±7.76	
Parathion-methyl	6.72±1.01	9.81±3.78	0.1	6.88±1.27	14.14±7.58	0.4	6.57±1.59	5.68±1.44	
Methidathion	3.31±0.49	3.07±0.50	0.1	3.10±0.79	3.10±0.77	0.4	3.52±0.56	3.04±0.66	
Phosalone	12.35±1.6	12.21±1.79	0.6	13.86±2.53	10.37±1.60	0.4	10.91±2.11	13.96±3.14	

Data were shown as mean±SD
Abbreviations: PD, Parkinson's disease; Con, control; α-HCH, α-hexachlorocyclohexane; β-HCH, β-hexachlorocyclohexane; γ-HCH, γ-hexachlorocyclohexane; δ-HCH, δ-hexachlorocyclohexane; p,p'-DDE, p,p'-dichloro-diphenyldichloroethylene; p,p'-DDD, p,p'-Dichlorodiphenyldichloroethane; o,p'-DDT, o,p'-dichloro-diphenyl-trichloroethane, p,p'-DDT, p,p'-dichloro-diphenyl-trichloroethane. *: P<0.05, **: P<0.01.

We also compared the association between clinical characteristics of PD patients and serum levels of pesticides. HAMD (Hamilton Depression Scale, HAMD), HAMA (Hamilton Anxiety Scale, HAMA) and MoCA (Montreal Cognitive Assessment, MoCA) scores differed among the Tertile 1, 2 and 3 groups for dieldrin (HAMD: 2.69±0.77, 5.70±1.11, 10.82±1.44, P<0.01; HAMA: 5.69±1.32, 7.30±1.41, 11.93±1.39, P=0.02; MoCA: 26.00±3.49, 21.97±0.92, 21.18±0.86, P=0.02). A multiple comparison test revealed that the HAMD score was significantly higher in the Tertile 3 group compared to Tertile1 and 2 groups (P<0.01 and P=0.02, respectively), while the MoCA score was lower in the Tertile 3 group than Tertile 1 group (P=0.02). After controlling for age and sex, serum level of dieldrin was positively associated with HAMD score and negatively associated with MoCA score in PD patients (P<0.01, P=0.04). It has been reported that exposure to pesticides was a risk factor for depression. A longitudinal study also found a link between exposure to dieldrin and depression [8]. We also observed that dieldrin was negatively associated with cognitive function. Although there were no reports of an association between dieldrin levels and MoCA score in PD patients, this compound has been linked to increased risk of AD. It remains to be determined whether specific pesticides, and especially dieldrin are associated with depression symptoms and cognitive function in PD.

Gene-environment interactions play an important role in PD. We did not find an association between 10 SNPs and the genetic susceptibility of PD (Table e-4), which might have been due to the small sample size (primers for the 10 SNPs were listed in Table e-5). After controlling for confounding factors such as sex, age and region, statistically significant interactions were found between rs11931074 polymorphism in the *SNCA* gene and α-HCH (OR: 2.08; 95% CI: 1.1-3.8; P=0.02), between rs11931074 in *SNCA* gene and β-HCH (OR: 2.08; 95% CI: 1.0-3.2; P=0.05), and between rs16940758 in *MAPT* (microtubule-associated protein tau, MAPT) gene and δ-HCH (OR: 2.48; 95% CI: 1.0-5.9, P=0.04), causing an increased risk for PD. This provides the first evidence of an interaction between pesticide levels and rs11931074 of the *SNCA* gene on PD risk, although such a relationship has been demonstrated for the rs3775423 polymorphism and pesticide exposure [9]. The *MAPT* gene primarily encodes Tau protein, which is implicated in PD. A study demonstrated that interaction between *MAPT* rs16940758 or rs2435211 and exposure to pesticides had no significant effect on PD, either [9]. *ABCB1* rs1045642 and *NOS1* rs12829185 were reported to interact with OCPs exposure to increase PD risk, which were not substantiated in our results. *CYP1A1* (cytochrome P450, family 1, subfamily A, polypeptide 1, *CYP1A1*), *CYP1B1* and *CYP2B6* are members of the cytochrome P450 family and are mainly involved in drugs and neurotoxins metabolism, although no association between their interactions and PD has been demonstrated, probably due to the limited sample size in this study.

Given that α-HCH and propanil levels were associated with increased risk of PD, we investigated the molecular basis for this observation using SH-SY5Y cells. We found that cell viability was decreased upon α-HCH or propanil stimulation (Fig. e-2A, e-2B). We also found here that α-HCH and propanil increased the production of ROS (Reactive oxidative stress, ROS) (Fig. e-2C, e-2D) while decreasing the mitochondrial membrane potential (ΔΨm) in neuronal cells (Fig. e-2E, e-2F). This is in accordance with the finding that α-HCH increased the ROS generation in keratinocytes and that propanil induced lipid oxidation in rat models. OCPs are known to decrease ΔΨm in neuronal cells, and this was confirmed for α-HCH and propanil in the present study. Additionally, we found that propanil, rather than α-HCH induced the aggregation of α-synuclein in neuronal cells. (Fig. e-2G, e-2H). Rotenone, another type of pesticide, was shown to have the same effect, and dieldrin was reported to accelerate the rate of a-synuclein fibrils formation in vitro [10]. However, we did not find that α-HCH significantly altered the aggregation of α-synuclein. Our results suggested that α-HCH might be involved in PD via oxidative stress or other pathways, which needs to be further investigated.

There were some limitations to the present study, including the relatively small sample size, limited mechanisms of α-HCH and propanil and contribution of interactions between genetic variants and pesticides into PD development. On the other hand, we included the healthy spouses of PD patients as controls to minimize the differences in serum pesticides levels caused by dietary factors and recruited participants from both urban and rural areas to reduce the effects

on the results. Finally, our findings provide the first demonstration of a correlation between specific pesticides in serum and PD and the first analysis of the interaction effect between specific pesticides and gene variants on PD in the Chinese population.

Conclusion

In summary, our results indicate that elevated serum levels of α-HCH and propanil are associated with increased risk for PD. We also showed that serum levels of dieldrin are associated with depression and cognitive function in PD patients and that the interaction between genetic variants and pesticides increased PD risk. Based on these findings, more stringent environmental regulations may need to be implemented to reduce PD risk in the population, especially in agricultural areas where communities may be exposed to unsafe pesticide levels.

Abbreviations

ABCB1: ATP-binding cassette, sub-family B, member 1;

α-HCH: α-hexachlorocyclohexane;

CYP1A1: cytochrome P450, family 1, subfamily A, polypeptide 1;

ΔΨm: Mitochondrial membrane potential;

HAMA: Hamilton Anxiety Scale;

HAMD: Hamilton Depression Scale;

MAPT: microtubule-associated protein tau;

MoCA: Montreal Cognitive Assessment;

NOS1: nitric oxide synthase 1;

OCPs: organochlorine pesticides;

o,p'-DDT: o,p'-dichloro-diphenyl-trichloroethane;

OPs: organophosphorus pesticides;

PD: Parkinson's disease;

p,p'-DDD: p,p'-dichlorodiphenyldichloroethane;

p,p'-DDE: p,p'-dichlorodiphenyltrichloroethane;

ROS: Reactive oxidative stress;

SNCA:α-synuclein;

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of medicine (No. 2017-8). All participants signed an informed consent.

Consent to publication

All the authors have approved the manuscript.

Availability of data and materials

Data are available upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by grants from the National Key R&D Program of China (Grant No. 2016YFC1306000), the National Natural Science Foundation of China (Grant Nos. 81801254, 81870998 and 81901283), the Key Field Research and Development Program of Guangdong Province (Grant No.

2018B030337001), the Shanghai Sailing Program (Grant No. 18YF1414000).

Authors' contributions

SX and XY performed the experiments, clinical analysis, and manuscript writing; YQ collected sample and extract DNA; DW and FS helped to recruit PD patients and controls. QL helped to detect the of serum pesticides. YS helped to perform the statistical analysis; QX performed the study design, recruitment of PD patients and controls, project management, financial support and manuscript revision. All authors meet the qualifications for authorship and have reviewed and approved the final version of the manuscript.

Acknowledgments

We are grateful to all the patients and controls for their generous participation in this study.

References

1. Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, et al. Pesticide exposure and risk of Parkinson's disease: a family-based case-control study. BMC Neurol. 2008;8:6.
2. Fleming L, Mann JB, Bean J, Briggle T, Sanchez-Ramos JR. Parkinson's disease and brain levels of organochlorine pesticides. Ann Neurol. 1994;36:100-103.
3. Richardson JR, Roy A, Shalat SL, Buckley B, Winnik B, Gearing M, et al. beta-Hexachlorocyclohexane levels in serum and risk of Parkinson's disease. Neurotoxicology. 2011;32:640-645.
4. Petersen MS, Halling J, Bech S, Wermuth L, Weihe P, Nielsen F, et al. Impact of dietary exposure to food contaminants on the risk of Parkinson's disease. Neurotoxicology. 2008;29:584-590.
5. Richardson JR, Shalat SL, Buckley B, Winnik B, O'Suilleabhain P, Diaz-Arrastia R, et al. Elevated serum pesticide levels and risk of Parkinson disease. Arch Neurol. 2009;66:870-875.
6. Weisskopf MG, Knekt P, O'Reilly EJ, Lyytinen J, Reunanen A, Laden F, et al. Persistent organochlorine pesticides in serum and risk of Parkinson disease. Neurology. 2010;74:1055-1061.
7. Chhillar N, Singh NK, Banerjee BD, Bala K, Mustafa M, Sharma D, et al. Organochlorine pesticide levels and risk of Parkinson's disease in north Indian population. ISRN Neurol. 2013;2013:371034.
8. Beard JD, Umbach DM, Hoppin JA, Richards M, Alavanja MC, Blair A, et al. Pesticide exposure and depression among male private pesticide applicators in the agricultural health study. Environ Health Perspect. 2014;122:984-991.
9. Chung SJ, Armasu SM, Anderson KJ, Biernacka JM, Lesnick TG, Rider DN, et al. Genetic susceptibility loci, environmental exposures, and Parkinson's disease: a case-control study of gene-environment interactions. Parkinsonism Relat Disord. 2013;19:595-599.
10. Uversky VN, Li J, Bower K, Fink AL. Synergistic effects of pesticides and metals on the fibrillation of alpha-synuclein: implications for Parkinson's disease. Neurotoxicology. 2002;23:527-536.

Figures

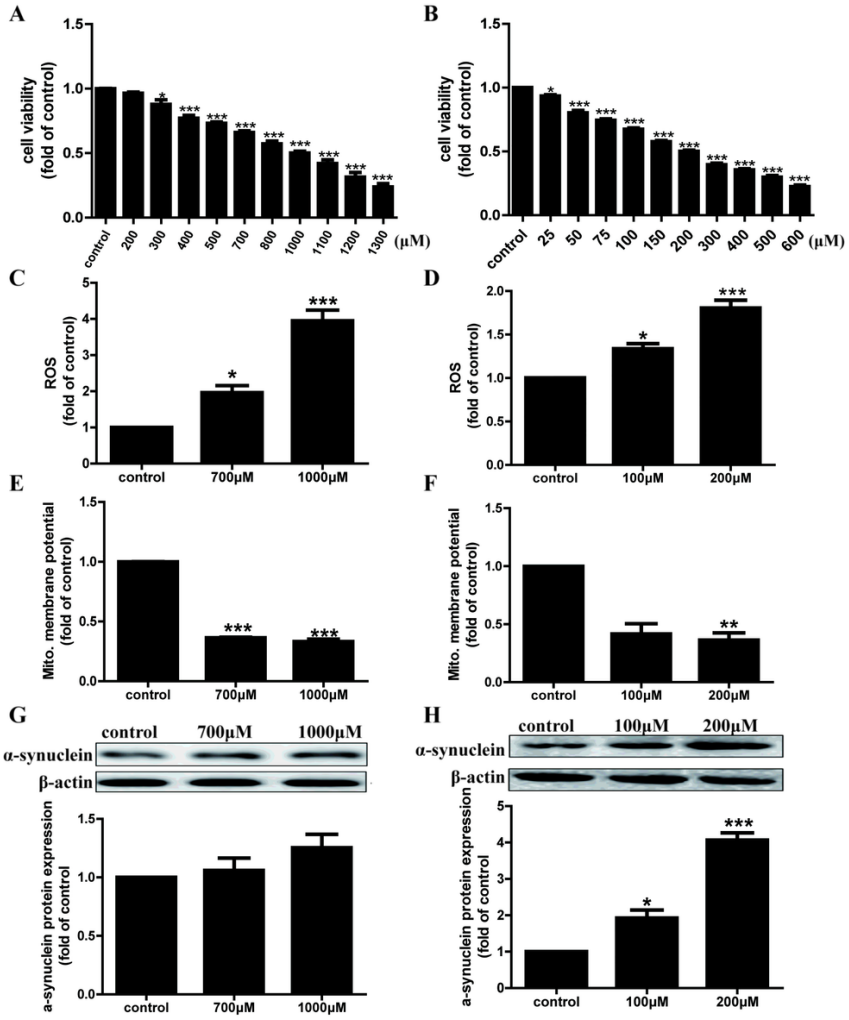

Figure 1

The effect of α-HCH and propanil on oxidative injury and α-synuclein aggregation in neuronal cells. (A, B) Cell viability of neuronal cells was decreased upon α-HCH (A) and propanil (B) stimulation for 48h detected by CCK-8. (C, D) ROS production was induced by α-HCH (C) and propanil (D) detected by CM-H2DCFDA. (E, F) The mitochondrial membrane potential was decreased by α-HCH (E) and propanil (F) stimulation detected by JC-10 fluorescent probe. (G) α-HCH had no significant effect on α-synuclein. (H) α-synuclein aggregation was enhanced by prapanil stimulation. α-HCH, α-hexachlorocyclohexane. Mito., mitochondrial. *: P≤0.05, **: P≤0.01, ***: P≤0.001 vs. control

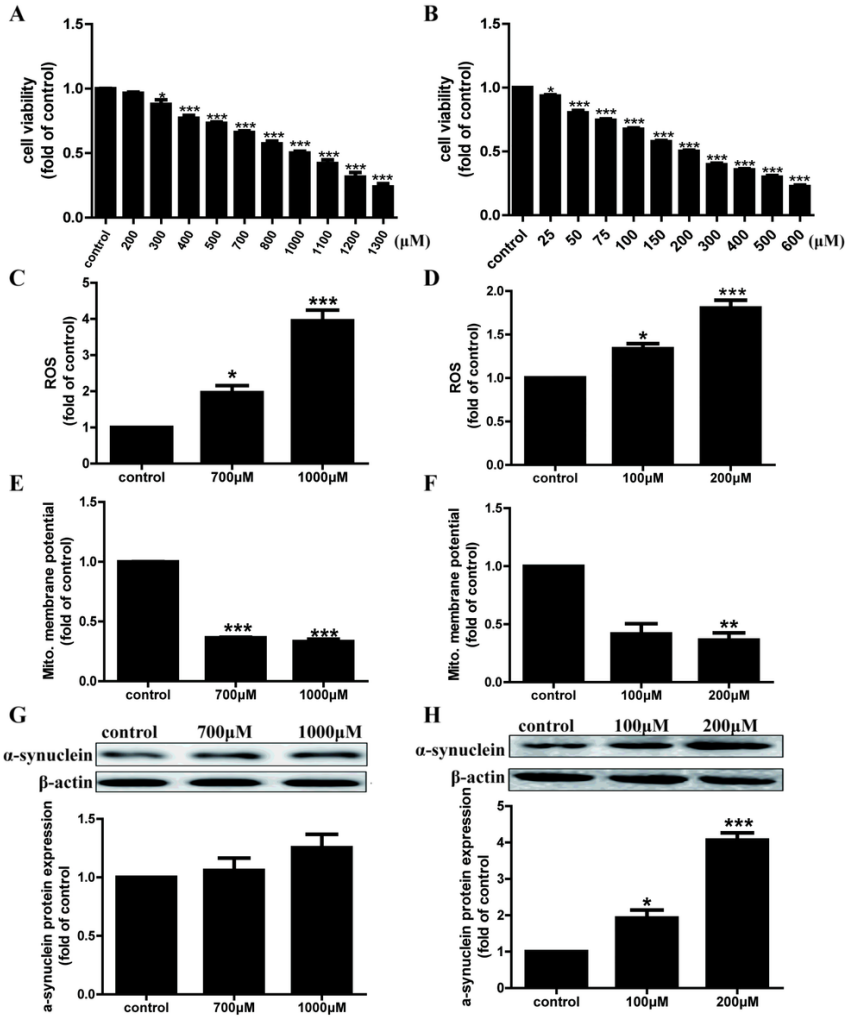

Figure 1

The effect of α-HCH and propanil on oxidative injury and α-synuclein aggregation in neuronal cells. (A, B) Cell viability of neuronal cells was decreased upon α-HCH (A) and propanil (B) stimulation for 48h detected by CCK-8. (C, D) ROS production was induced by α-HCH (C) and propanil (D) detected by CM-H2DCFDA. (E, F) The mitochondrial membrane potential was decreased by α-HCH (E) and propanil (F) stimulation detected by JC-10 fluorescent probe. (G) α-HCH had no significant effect on α-synuclein. (H) α-synuclein aggregation was enhanced by prapanil stimulation. α-HCH, α-hexachlorocyclohexane. Mito., mitochondrial. *: P≤0.05, **: P≤0.01, ***: P≤0.001 vs. control

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarymaterialsrevised.pdf
- supplementarymaterialsrevised.pdf