J Appl Biotechnol Rep. 2024 March;11(1):1236-1244

Journal of
Applied Biotechnology Reports

doi 10.30491/JABR.2024.193563

Original Article

Evaluation of the Protective Effect of Organophosphorus Hydrolase on SH-SY5Y Human Neuroblastoma Cells Treated with Parathion

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Received February 19, 2023; **Accepted** October 20, 2023; **Online Published** March 15, 2024

Abstract

Introduction: Organophosphorus (OPs) compounds are chemical compounds used in pesticides that contain synthetic esters, amides, and thiol derivatives of phosphoric, and phosphonic acids. The OPs are harmful to humans and animals because of compounds such as parathion. By acting on nerve cells, parathion creates very dangerous cellular oxidative stresses, which in turn activate programmed cell death.

Materials and Methods: In this study, the enzyme Organophosphorus Hydrolase (OPH) having esterase activity was selected with the aim of influencing its reaction product with parathion on the viability of human nerve cells. The neuroblastoma SH-SY5Y cell line was exposed to parathion (700 µg/ml) (≈ 40% reduced cell viability) and the product of OPH esterase reaction (1 µg/ml) with the same parathion concentration for two hours to determine their cytotoxicity (≈ 25% reduced cell viability) by MTT, real-time PCR and flow cytometry techniques.

Results: The results revealed that parathion (100 µg/ml) inhibited acetylcholinesterase activity by ≈ 65% while OPH-related product reduced acetylcholinesterase activity by ≈ 26%.

Conclusions: Considering the widespread use of OPs in modern agriculture, the OPH can be used to reduce the OPs' destructive effects and the current study could provide new insight into healthy modern agriculture.

Keywords: Organophosphorus, Organophosphorus Hydrolase, Parathion, Acetylcholine esterase

Citation: Mirzaei M, Moosazadeh Moghaddam M, Seidmoradi R, Rastegar Shariat Panahi M, Latifi AM. Evaluation of the Protective Effect of Organophosphorus Hydrolase on SH-SY5Y Human Neuroblastoma Cells Treated with Parathion. J Appl Biotechnol Rep. 2024;11(1):1236-1244. doi:10.30491/JABR.2024.193563

Introduction

Agricultural chemicals and pesticides have generally been important component of agricultural systems around the world over the past century, and these compounds significantly increase crop yields and food production.[1] Different studies determined that they have toxic effects on biologic systems.[2,3] Organophosphorus (OPs) compounds are kinds of pesticides that extensively are utilized in agriculture. OPs are harmful to human beings and animals because of their chemical compounds. They are commonly more toxic than other kinds of insecticides,[4,5] Parathion is a highly toxic organophosphate (OP) pesticide. World Health Organization (WHO) and the United States Environmental Protection Agency (U.S. EPA) reported it as an extremely toxic material in the world (U.S. EPA, 1999).[6] Agricultural field is where that people or animals are exposed to OPs and[7] respiratory routes, oral, and dermal are the ways of parathion absorption. Acetylcholine (ACh) accumulation at the neuronal synapses and neuromuscular junctions, when acetylcholinesterase's (AChE) activity is blocked by OPs and put humans and animals in dangerous situations and can ended to death.[5] Blocking acetylcholinesterase activity in the neuron system is the way that parathion affect. In fact, Ability to bind to acetylcholinesterase (AChE) and put stop to the hydrolysis of acetylcholine (ACh) by the active oxon metabolite in both insects and humans is the primary toxicity of Parathion.[8,9] Parathion induces oxidative stress in cells and continuance to disorder neurotransmission and inhibits cells division in the nervous system.[10–12] *In vitro* studies on PC12 cells as a standard model for neural cell differentiation showed that Organophosphate insecticides cause an increase in Reactive Oxygen Species (ROS) after OPs exposure to the PC12 cell line.[13] In fact, OPs reduced mitochondrial activity in PC12 cells, then, the formation of ROS increased in mitochondria. ROS starts signals and cytochrome c release from mitochondria, the cytochrome c signals activate apoptotic cell death,[14] Parathion is an OPs that induces

apoptotic cell death in the neuroblastoma SH-SY5Y cell line too.[15] These effects have been relative to imbalance proteins expression such as Bcl2 and Bax in cells.[16–18] The Bcl2 protein expression is anti-apoptotic and the Bax protein expression is a pro-apoptotic signal.[19] Parathion released cytochrome c that causes Caspase-3 activation and induced apoptosis in SH-SY5Y. After that, nuclear DNA condensation and fragmentation will happen. Finally, apoptotic cell death occurs by Caspase-3 activation.[20] Other mechanisms of action are being investigated that increased concerns about the harmful biological effects of exposure to Parathion. Organophosphorus Hydrolase (OPH) is an enzyme that can degrade OPs, its products are another toxic, p-nitrophenol, with low toxicity and some none toxic compounds.[21,22] *Pseudomonas Diminuta* and *Flavobacterium species* are some soil microorganisms, which naturally have OPH.[23] OPH has specific substrates, such as P─O, P─CN, P─F, and P─S. As a strong biocatalyst, OPH can hydrolysis the OPs, so it was suggested as a degradation enzyme for Ops.[21,24] In this study, we chose OPH to degrade Parathion to observe neuroblastoma cell line (SH-SY5Y) apoptosis and acetylcholinesterase activity. For this regard, MTT assay, Real-Time PCR, and Flowcytometry were used.

Materials and Methods

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[25] The Sh-y5y human neuroblastoma cells (CRL-2266) were obtained from Pasteur Institute National Cell Bank of Iran (Iran). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin all were purchased from Gibco (Gaithersburg, MD, USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) was purchased from sigma (Sigma-Aldrich, St. Louis, MO, USA). cDNA kit was purchased from PCR Biosystem (England). SYBR Green qPCR kit was obtained from Qiagen (USA). FITC annexin-V/dead cell apoptosis kit was obtained from Biolegend (England). Hoechst 33258 was purchased from Thermo Fisher Scientific (USA). DMSO was purchased Sigma (USA), RNA extraction kit was obtained from Yektatajhiz (Iran) Parathion Sigma (USA). In this study we used the OPH in previous study.[26]

Cell Culture

The Sh-y5y human neuroblastoma cell line were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 µg/ml streptomycin, at 37 °C in a humidified atmosphere of 5% CO_2. In the experiment, cells were seeded onto appropriate culture plates. The cells counting was done by trypan blue staining. The cultured cells were seeded and incubated (in 37 °C / 5% CO_2) for 1 day to adhering on the plates.

Cell Viability Measurement by MTT

The 5×10^3 cells/well of Sh-y5y were seeded onto a 96-well plate and incubated (in 37 °C / 5% CO_2) for 1 day. To determine viability of the Sh-y5y cells against Parathion by MTT assay, the cells were treated by Parathion concentrations of 350, 700, and 1400 µg/ml in 0.1% DMSO-containing DMEM for 15, 30 min, and 1, 2, 4, and 6 hours. After removing supernatants, 20 µl of MTT solution (5 mg/ml in PBS) was added and the cells were incubated for 4 hours. The medium throwed out, and the formazan dissolved in 20 µl of DMSO. The color reaction was measured at wavelength 570 nm by BIO RAD spectrophotometer (England). Considering the best Parathion concentration and treatment time, various OPH concentrations (500 ng/ml, 1 µg/ml, and 2 µg/ml) were prepared by adding OPH to Parathion solution (700 µg/ml), the Sh-y5y cells were treated for 2 hours, and cellular viability was measured by using MTT assay.

Quantitative real time-PCR (qPCR) Analysis

The 9×10^4 cells/well of Sh-y5y were seeded onto a 6-well plate and incubated (in 37 °C, 5% CO_2) for 1 day. Then the cells were treated with Parathion solution (700 µg/ml in 0.1% DMSO-containing culture media), and OPH solution (1 µg/ml in Parathion solution) for 2 hours. After removing the medium, trypsinized, and centrifuged at 1500 rpm for 5 min. Total RNA was extracted RNA extraction kit according to the supplier's instructions, and quality and purity of the extracted RNA were determined at absorption ration of A_{260}/A_{280}. In the following, the cDNA was synthesized using the cDNA synthesis kit, and qPCR was done (SYBR GREEN qPCR kit) using StepOne ABI applied Biosystem (USA). The sequences of forward and reverse primers were presented in Table 1. GAPDH was used as a reference gene, and gene expression was determined by the $2^{-\Delta\Delta Ct}$ method.

Table 1. Sequences of Forward and Reverse Primers for qPCR Analysis

Genes	Primer Sequence	
Bax	Forward	5-CCCGAGAGGTCTTTTTCCGAG-3
	Reverse	5-CCAGCCCATGATGGTTCTGAT-3
Bcl2	Forward	5-CATGTGTGTGGAGAGCGTCAA-3
	Reverse	5-GCCGGTTCAGGTACTCAGTCA-3
GAPDH	Forward	5-TCAAGGCTGAGAACGGGAA-3
	Reverse	5-TGGGTGGCAGTGATGGCA-3

Flowcytometric Detection of Apoptotic Cells

The Sh-y5y cell line (9×10^4 cells/well) was seeded on a 6-well plate and incubated (in 37 °C, 5% CO_2) for 1 day. Then treated by Parathion solution (700 µg/ml in 0.1% DMSO-containing culture media), and OPH solution (1 µg/ml in Parathion solution) for 2 hours. Then centrifuged at 1500 rpm for 5 minutes removed the medium and washed the cells two times with PBS. Then the cells suspended in tubes at a

concentration 1 × 10^5 cells/ml. The FITC annexin-V/dead cell apoptosis kit was used to determined apoptotic cells. So, 100 µl of Annexin V Binding Buffer added to cells and incubated them with 10 µl of FITC annexin-V and 5 µl propidium iodide (PI) at room temperature for 15 minutes. FACS Calibur flow cytometer instrument used to measuring apoptotic cells. The fluorescence emission determined and the annexin-V-positive and PI-negative (early apoptotic) and annexin-V-positive and the PI-positive (late apoptotic) were analyzed.

Staining of Nuclear DNA

Sh-y5y cells were seeded on a 24-well plate and treated with Parathion solution (700 µg/ml in 0.1% DMSO-containing culture media), and OPH solution (1 µg/ml in Parathion solution) for 2 hours. Then, the cells fixed with 4% paraformaldehyde in 1X PBS (pH = 7.4) for 20 minutes at room temperature (RT) and washed each well three times with PBS. The cells were stained with 1mg/ml Hoechst 33258 in PBS for 20 minutes at RT and then washed again. The changing of the nuclei morphology of apoptotic cells can be observed because the Hoechst 33258 binding to triplet adenine and thymine base pairs in the minor groove outside the double helix.

Measuring Acetylcholinesterase Activity

Sh-y5y cells were seeded on a T-75 cell culture flask and incubated (in 37 °C / 5% CO_2) for 1 day. Sh-y5y cells reached 50–60% confluency. For maximize basal AChE activity, the medium (10 ml) mixed with retinoic acid (20 µM, final concentration) for 7 days. The number of 2 × 10^6 cell was incubated at 37 °C for 1 hour with samples including: Parathion solution (100 µg/ml in 0.1% DMSO-containing PBS), OPH (1 µg/ml in Parathion solution), OPH (1 µg/ml in PBS), and 0.1% DMSO-containing PBS as negative control. Then, the cells were centrifuged at 1500 rpm for 5 minutes, washed with PBS for 2 times, and resuspended in 200 µl PBS. In the following, the cells were sonicated on ice for 5 seconds for disruption, and centrifuged at 9000 rpm (4 °C) for 20 minutes. A volume of 25 µl of each sample's supernatant was added to 225 µl of a substrate mixture (125 µl 100 mM sodium phosphate buffer (pH 8) + 50 µl 10 mM acetylthiocholine + 50 µl 10 mM dithiobisnitrobenzoate (DTNB) in 100 mM sodium phosphate buffer (pH 7) containing 18 mM sodium bicarbonate + 0.1 mM ethopropazine). Finally, the tubes were incubated at 37 °C for 30 min, and enzyme activity was measured by spectrophotometry at 405 nm.[27]

Statistical Analysis

All experiments of MTT were performed in quintuplicate, qPCR and Flow cytometry in duplicate, and acetylcholinesterase activity in sextuplicate. Statistical analyses were done using one-way ANOVA test followed by Tukey's post hoc analysis at significant level of p <0.05. All data were presented as mean ± standard deviation (mean ± SD).

Results

Effect of Parathion, OPH, and Parathion + OPH on Sh-y5y Cells Viability

Sh-y5y Cells treated with Parathion degradation by OPH had better viability compare treated with Parathion alone (Figure 1c). Sh-y5y treated with Parathion by Parathion concentrations of 350, 700, and 1400 µg/ml in 0.1% DMSO-containing DMEM for 15, 30 min, and 1, 2, 4, and 6 hours. And OPH (500 ng/ml, 1 µg/ml, and 2 µg/ml) for 2 hours. Cell viability reduced with Parathion (700 µg/ml, and 1400 µg/ml in 0.1% DMSO-containing DMEM) and OPH (2 µg/ml). Cell viability compared with control (p <0.01) decreased ≈ 40% when they were treated with Parathion (700 µg/ml in 0.1% DMSO -containing DMEM) and decreased ≈ 25% when cells were treated with OPH (2 µg/ml) for 2 hours. (Figures 1a and 2a). Based on these results, 1 µg/ml OPH and 700 µg/ml Parathion were chosen in ahead experiments. Sh-y5y cells were exposed to Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) in the presence of OPH (1 µg/ml) for 2 hours, and cell viability increase with co-treated (Parathion + OPH) cells compared to the sample treated with Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) about 15% (Figure 1c).

Effect of Parathion and Parathion + OPH on Bax and Bcl-2 mRNA Expression

Real-time quantitative RT-PCR was used to determine expression of mRNA of Bax and Bcl-2 (Figure 2a and 2b). Treated cells by Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) showed increase in Bax expression compared to that of control cells (Figure 2a and 2b). Cells treated with (700 µg/ml in 0.1% DMSO-containing DMEM) in the presence of OPH (1 µg/ml) showed decreased the Bax expression, compared to Parathion (700 µg/ml) but increased the Bax expression compared to control (p <0.01) (Figure 2a). Cells treated by Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) showed decreased Bcl-2 expression, compared to control. And SH-SH5Y Cells treated with Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) with OPH (1 µg/ml) increased Bcl-2 expression, compared to Parathion (700 µg/ml) and decrease compared to control. (p <0.01) (Figure 2b).

Determine effect of Parathion and Parathion + OPH on Apoptosis

The flow cytometry technic with PI and annexin-V dual staining was used to quantified apoptotic cells. Normal cells were determining with negative annexin-V/negative PI, and to consider early stage of apoptosis cells used of positive

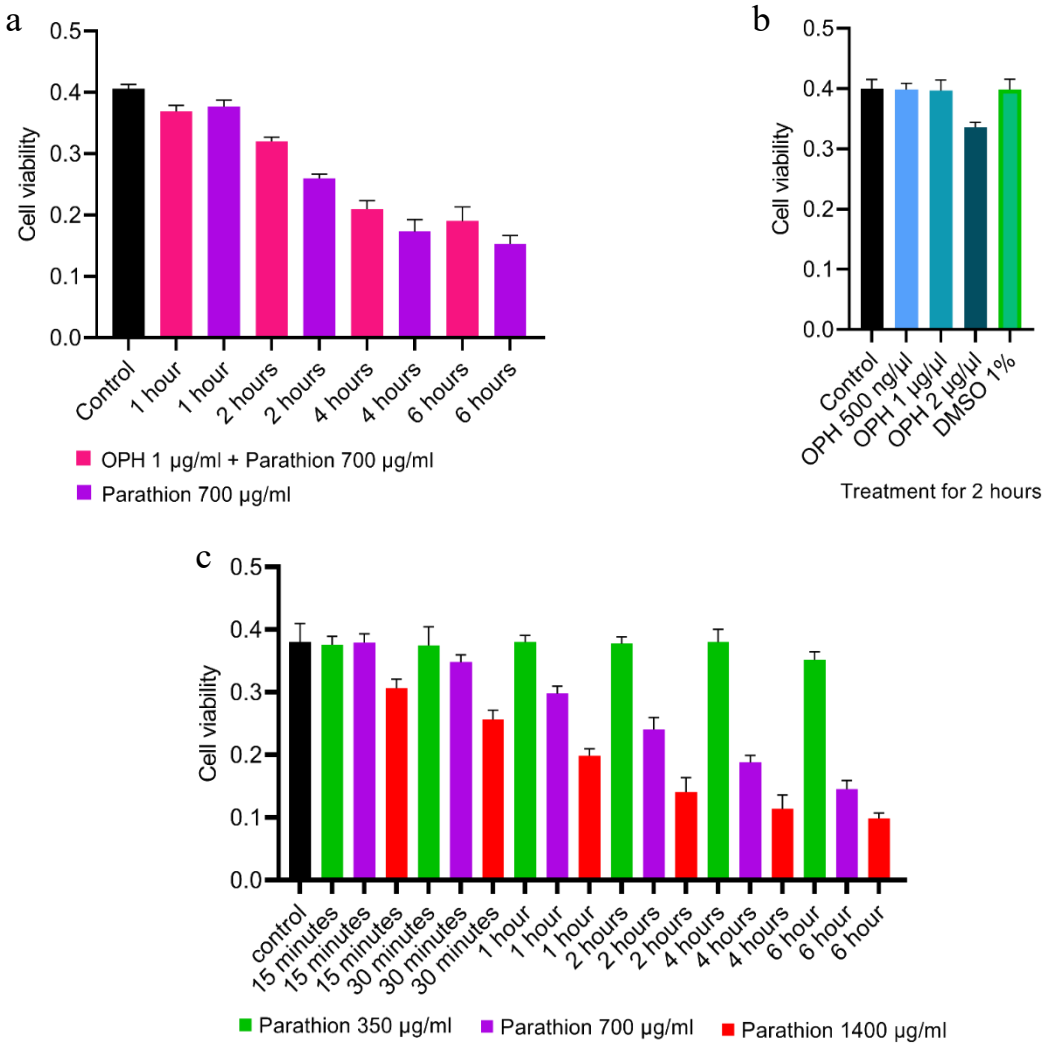

Figure 1. a) Toxicity of Parathion in (350 µg/ml, 700 µg/ml, and 1400 µg/ml) Concentrations in SH-SY5Y Viability; **b)** Toxicity of OPH (500 ng/ml, 1 µg/ml, and 2 µg/ml), and DMSO 0.1% after 2 hours in SH-SY5Y Viability; **c)** Toxicity of Parathion solution (700 µg/ml in 0.1% DMSO-containing DMEM) in Present of OPH (1 µg/ml) and Parathion (700 µg/ml) after 2 hours in SH-SY5Y viability. All the samples' toxicity in this stage measuring by MTT assay.

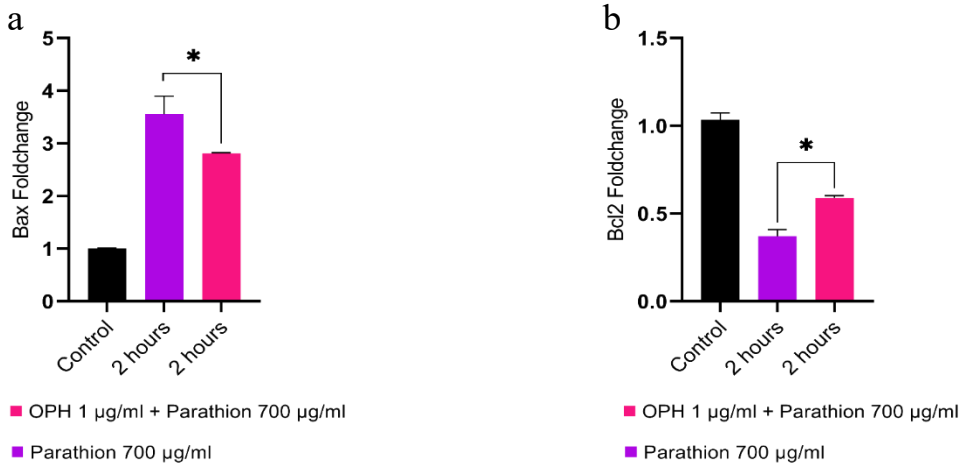

Figure 2. Effect of Parathion and OPH + Parathion Reaction on Bax and Bcl-2 mRNA Expression in Sh-y5y Cells. Cells were treated with Parathion solution (700 µg/ml in 0.1% DMSO-containing DMEM) in present of OPH (1 µg/ml) + OPH (1 µg/ml) and Parathion (700 µg/ml) for 2 hours. Expression of Bax **(a)** and Bcl-2 **(b)** was analyzed with quantitative real-time RT-PCR, and compared to their untreated controls. The expression levels of the target gene were estimated by the $2^{-\Delta\Delta Ct}$ method after normalizing to the expression level of GAPDH. Data are expressed as mean as mean ± SD (n = 3). $^{*}p<0.01$; $^{**}p<0.001$.

annexin-V/negative PI, while positive annexin-V/positive PI were considered to determine the necrosis/late apoptosis stage. Figure 3a, 3b, and 3c) Sh-y5y cells treated with Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) for 2 hours and apoptotic cells' percentage increased compare untreated cells (p <0.001; Figure 3b and 3d). And production between Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) (Figure 3b, 3c, and 3d) and OPH (1 µg/ml) decreased apoptotic cells compare the cells treated with Parathion (700 µg/ml) alone, while increased apoptotic cells compare the untreated cells (Figure 3b and 3d) (p <0.001).

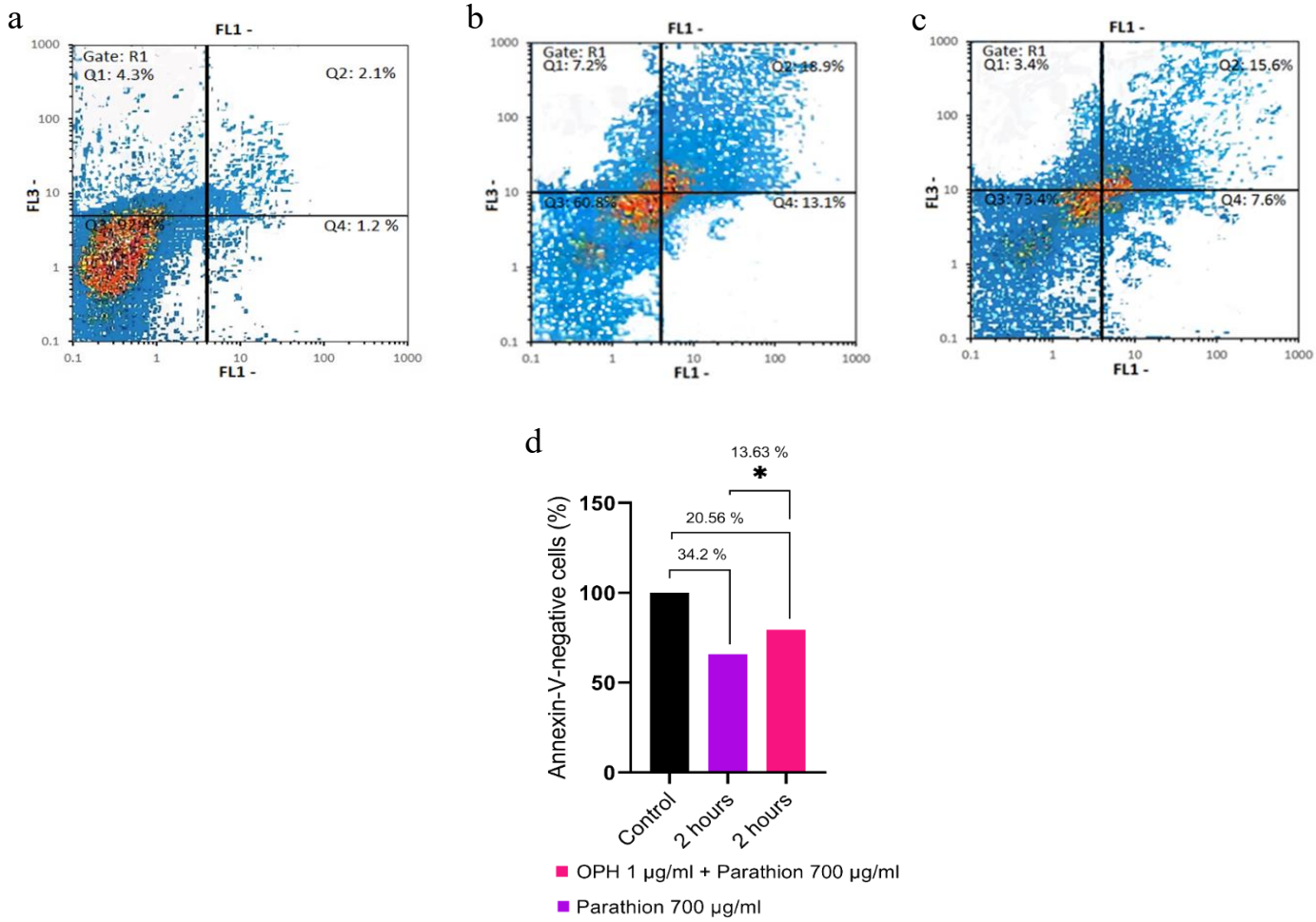

Figure 3. Effect of Parathion and OPH + Parathion Induced Apoptosis in Sh-y5y Cells. Cells were exposed to with Parathion solution (700 µg/ml in 0.1% DMSO-containing DMEM) in present of OPH (1 µg/ml) with OPH (1 µg/ml) and Parathion (700 µg/ml) for 2 hours. Apoptosis was evaluated by staining cells with annexin-V. **a)** Control; **b)** Parathion (700 µg/ml in 0.1% DMSO-containing DMEM); **c)** Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) in present of OPH (1 µg/ml). d) Percentage of annexin-V-positive cells was calculated. Data are expressed as mean ± SD (n = 3) of percentage to untreated cells. *p <0.001.

Characterized of Apoptosis Nuclear Morphology

Hoechst 33258 staining was using to Characterize of apoptosis nuclear morphology. The apoptotic nuclei were determined to shape condensed or fragmented nuclei. Cells treated with Parathion (700 µg/ml in 0.1% DMSO-containing DMEM) OPH (1 µg/ml) decreased the number apoptotic cells compare the cells treated with Parathion (700 µg/ml). (Figure 4d, 4e) and both kinds of treatments show increase apoptotic cells compare the untreated cells. (p <0.001; Figure 4d, 4e, and 4f).

Measuring AChE Activity in SH-SY5Y Cells

SH-SY5Y cells' AChE activity were inhibiting with Parathion (100 µg/ml) after 1 hour incubation, and Parathion (100 µg/ml in 0.1% DMSO-containing PBS) reaction with OPH (1 µg/ml) incubation for 1 hour, then treated cells with parathion (100 µg/ml in 0.1% DMSO-containing PBS) + OPH (1 µg/ml) for 1 hour reduced AChE inhibition in SH-SY5Y cells. SH-SY5Y cells' AChE activity weren't changing with OPH (1 µg/ml) and PBS 1X. The AChE activity in SH-SY5Y significantly blocked (65%) with Parathion (100 µg/ml), while OPH (1 µg/ml) increased AChE activity compare to Parathion (100 µg/ml) alone and AChE activity reduced just 26% compare to untreated AChE

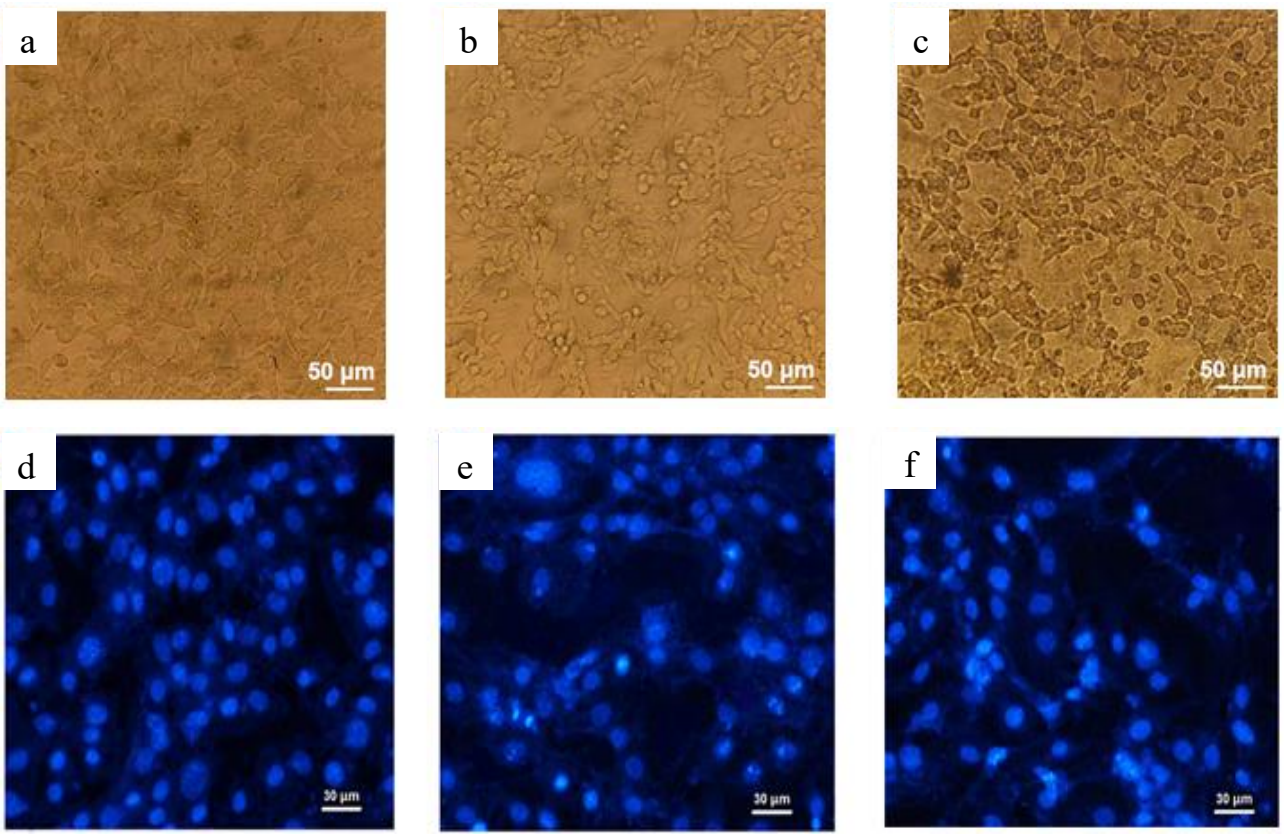

Figure 4. Phase-Contrast Images (a, b, and c) and Apoptotic Nuclear Morphology DNA Staining with Hoechst 33258 (d, e, and f) of Sh-y5y Cells Exposed to Parathion and OPH + Parathion Reaction. **a, d)** Control; **b, e)** Parathion solution (700 µg/ml in 0.1% DMSO-containing DMEM) in present of OPH (1 µg/ml) for 2 hours; **c, f)** Parathion (700 µg/ml) 2 hours and. cell viability was assessed with phase-contrast microscopy.

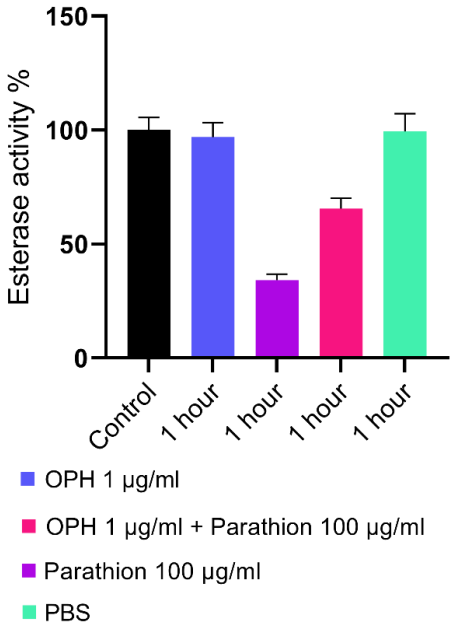

Figure 5. Acetylcholinesterase (AChE) Activity in Sh-y5y Cells Exposed to Parathion Solution (100 µg/ml in 0.1% DMSO-containing PBS), OPH (1 µg/ml in Parathion solution), OPH (1 µg/ml in PBS), and 0.1% DMSO-Containing PBS as Negative Control.

in SH-SY5Y cells (Figure 5). AChE activity in SH-SY5Y cells restored by treated OPH (1 µg/ml) (Figure 5).

Discussion

OPs are large group of synthetic compounds, including esters,

amides, thiol derivatives of phosphoric, phosphonic, phosphorotioic, and phosphonotioic-acids. Previous studies confirmed that Ops are toxic for human.[28] OPs (most insecticides, e.g., Parathion, Malathion, Chlorpyrifos, Diazinon, Dichlorvos) acts as transformers of acetylcholinase (hibylcholine) at the synaptic.[29] Most chemical and physical methods for the decomposition of organophosphate compounds have many defects and problems, these methods are often toxic, allergenic, corrosive, nonspecific, and harmful to the environment, animals, and human.[30] Enzyme isolated from microorganisms called OPH, have been identified that are capable of degrading organophosphate compounds.[31–33] Different studies reported OPs (Parathion, Chlorpyrifos, etc) induce apoptosis in SH-SY5Y human neuroblastoma cells and the cells of rat prefrontal cortex.[34–36] In this study for the first time we demonstrated the apoptosis effect of OPH-degraded Parathion in SH-SY5Y cells.

According to a study parathion solution (100 µg/ml) after 24 hours decrease Shsy5y viability about 50%.[37] In this study SH-SY5Y cells were exposed to Parathion solution (700 µg/ml in 0.1% DMSO-containing DMEM) and Parathion solution in the presence of OPH (1 µg/ml) for 2 hours, the MTT assay results showed increase (≈ 15%) in cell viability compare to cells that were treated with Parathion solution alone. According to the this result the OPH can reduce the Parathion toxicity on SH-SY5Y cells, actually OPH degradation Parathion and the product of their reaction have lower toxicity in treated cells. According to real-time results, Parathion solution and Parathion solution with OPH (1 µg/ml) imbalanced Bcl2 and Bax gene expression. In this stage, Parathion solution triggered apoptotic path way in SH-SY5Y with decrease in Bcl2 expression (3.2 fold change) and increase in Bax expression (3.5 fold change) compare to untreated cells. Parathion solution in present of OPH (1 µg/ml) lead to increase in Bcl2 expression (0.45 fold change) less than treated cells with Parathion solution alone. Bax, another pre apoptotic gene, has different expression level in SH-SY5Y cells that were treated with Parathion solution and Parathion degraded by OPH. Its expression increased (0.26 fold change) during treatment with Parathion solution in presence of OPH (1 µg/ml) compare to treatment with Parathion solution alone. The cell apoptosis process is the gene irregulated mechanism,[38] and OPH can affect on pre apoptotic genes expression in SH-SY5Y treated with Parathion solution and improve their expression level.

The Bcl-2 and Bax genes trigger apoptosis in cells through controlling the release of cytochrome c as an apoptosis factor from mitochondria. Bcl-2 and Bax lead to flip flop of the phosphatidylserine from the internal plasma membrane to external plasma membrane as well.[39–42] During apoptosis, annexin-V can bound with phosphatidylserine exist on the external plasma membrane.[43] Nuclear condensation and DNA cleavage occurring in the cell's apoptotic events relate to the mitochondria.[40] Our DNA staining and PI/annexin-V dual staining results confirmed that OPH plays an important role in Parathion degradation which lead to reducing the apoptosis in Sh-y5y neuroblastoma cells during treatment with Parathion solution.

Parathion acts as a neuron system toxic, actually it inhibits the cholinesterase activity in human and animal neuron systems, therefore acetylcholine accumulate at the neuronal synapses.[5,44] AChE activity is inhibited when its P─S bond changes to P─O bound,[27] and parathion can make this reaction happen.[45] In a study parathion (100 µg/ml) inhibited AchE activity about 70% and OPH returned AchE activity about 100% compared to untreated AchE.[27] For this reason, we used OPH (1 µg/ml) that was incubated with parathion (100 µg/ml) for 1 hour which leads to decrease in AchE inhibition.

The studies about parathion half-lives in pregnant rats, showed that the compound and metabolites relative to it, were detected (after analyzed) in some of the maternal and fetal tissues specially in adipose tissue, liver, and kidney, while the longest elimination half-lives were in liver, placenta, and brain.[46,47] According to our results, OPH can reduced the parathion toxicity in Sh-y5y neuroblastoma cells, and this enzyme has ability to use as a degradation agent of parathion or may other OPs *in vivo*. Overall, this study confirms the possibility of using OPH to degrade Parathion and reduced apoptosis in Sh-y5y neuroblastoma cells. These results will contribute to the development of remediation technology for reduced Parathion toxicity *in vitro*.

Conclusion

This study showed that OPH can decrease Parathion's cell cytotoxicity and apoptosis by degraded the Parathion in SH-SY5Y human neuroblastoma cells. Parathion also inhibits the cholinesterase activity in human and animal neuron systems. On the other hand, cholinesterase activity restored by OPH with degraded the Parathion. It can reduce the sides effects of Parathion on human and animal health. OPH is an appropriate recombinant protein to use decrease side effects of organophosphorus insecticides (Parathion) in poisonings caused.

Authors' Contributions

MAL participated in the study design. MM, MMM, RS, and MRSP contributed to all experimental work, data and statistical analysis, and interpretation of data reviewed the literature for the manuscript. M, HMH, and MB contributed extensively to the interpretation of the data and the conclusion. MAL, MM, and MMM performed the writing and editing of the final version of the manuscript. All authors approved the final draft for submission.

Ethical Approval

The study was confirmed by the Ethics Committee of the Baqiyatallah University of Medical Sciences (IR.BMSU. REC.1399.314).

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

Acknowledgment

The authors appreciate the efforts of colleagues in the laboratories of Baqiyatullah University of Medical Sciences.

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