Evaluation of Four Herbicides Cytotoxicity on Normal Liver THLE2 Cells

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The widespread increase in pesticide use is a source of pollution worldwide. Although herbicides have various applications, they nonetheless represent a risk to human health due to their tendency to bioaccumulate in many different environments. The purpose of this research was to investigate the cytotoxic effects of pendimethalin (PEN), oxyfluorfen (OFN), fluazifop-p-butyl (FPB), and pyraflufen ethyl (PFE) herbicides on human normal liver (THLE2) cells through the evaluation of the cell viability (using the MTT assay), apoptosis (using qPCR), cell cycle (using flowcytometry), and oxidative stress status (using biochemical assays). The four herbicides induced cytotoxic effects on THLE2 cells as evidenced by suppressed cell viability with IC50 values as follows: PEN (60.50±3.19 μM), OFN (100.36±4.66 μM), FPB (174.90±6.54 μM) and PFE (186.72±6.82 μM). They also increased apoptosis (high Bax and caspase 3, and low Bcl2 gene expression). However, only PEN and OFN induced cell cycle arrest in G0/G1 and both S and G2/M phases, respectively, with subsequent downregulated expression of cyclin D1, cyclin A2, and cyclin-dependent kinase 4 (CDK4). Among the four herbicides, only OFN, PFE, and FPB induced oxidative stress damage as revealed by higher levels of intracellular reactive oxygen species (ROS) and malondialdehyde (MDA), inhibited activities of antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)], and downregulated expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) genes. FPB and PFE had a greater safety margin on human normal THLE2 cells and might be used with less adverse consequences on human and animal health.

Keywords: Pendimethalin, Oxyfluorfen, Pyraflufen ethyl, Fluazifop-p-butyl, Cyclin-dependent kinase, Cytotoxicity.

Introduction

In many countries, the onion (Allium cepa L.) is a significant vegetable crop grown for export and domestic consumption [1,2]. It is grown across the world's tropics and subtropics when proper conditions exist [3,4]. Weeds significantly lower the crop's yield, quality, and value by competing with A. cepa for space, nutrients, light, and water [5,6], leading to substantial yield losses that have been reported to reach as high as 70-75% [7]. Several ways to get rid of weeds have been mentioned, such as using cultural, mechanical, herbicidal, and mulches [8]. Allium cepa weed population was greatly decreased by the use of pendimethalin (PEN), oxyfluorfen (OFN), fluazifop-p-butyl (FPB), and pyraflufen ethyl (PFE) herbicides, and A. cepa yield was enhanced [9,10]. The bad side of herbicides is their hazardous effects on not only plants but also humans and the environment. The Environmental Protection Authority recommends using herbicides that have a low impact on both humans and the environment.
Agency of the United States (USEPA) has placed PEN in toxicity class 3, while the US Environmental Protection Agency's classified it as a category C (human potential carcinogen) substance. PEN can trigger DNA damage-dependent cell death in Chinese hamster ovary (CHO) cells [11,12], rat hepatocytes, and thyroid FRTL 5 cells [13,14], and mouse bone marrow cells [15]. PEN is also a pollutant and is very hazardous to fish and other aquatic invertebrates [16] and has cytotoxic effects on human umbilical vein endothelial cells (HUVECs) through induction of cell cycle arrest in the G0/G1 phase [17,18]. Inducing genetic damage by herbicides may raise the occurrence of hereditary illness in future generations while also contributing to somatic cell disorders such as cancer in the current generation [11]. Indeed, cancer rates have been shown to rise by PEN exposure [19].

OFN kills weeds by inhibiting the enzyme protoporphyrinogen oxidase (PPO), which oxidizes to protoporphyrin IX when exposed to light and oxygen, generating reactive oxygen species (ROS) that may cause cell death [20]. OFN main side effects include an increased incidence of liver cancer in mice, inhibition of the protoporphyrinogen IX oxidase enzyme in rats, and a possible carcinogenic influence in humans [21]. It also has ROS-dependent cyto-and geno-toxic effects on fish and other aquatic invertebrates [22-26]. In zebrafish, OFN disrupts hepatic lipid and glucose metabolism during embryonic development and causes hepatocyte mortality by increasing inflammatory factors [27]. OFN induces promotes cell cycle arrest through the regulation of MAPK, PI3K, and autophagy in bovine mammary epithelial cells [28]. PFE, like OFN, prevents weed growth by inactivating the PPO, but it has a much stronger, more immediate herbicidal impact and is hardly hazardous to animals at all [29]. FPB is readily absorbed via leaf surfaces and is mostly converted to fluazipof-p (FP), which inhibits lipid formation through the inactivation of acetyl coenzyme A carboxylase and induces ROS overproduction resulting in lipid peroxidation and damage of cell membrane and finally cell death of weeds [30]. FPB triggers a cytotoxic effect on mammalian cells by the same mechanism [31]. Rats exposed to FPB showed signs of liver, kidney, and testis damage caused by oxidative stress [32]. Based on the previously mentioned data, little is known regarding the comparison between the deleterious effects of the four herbicides on normal human cells. Therefore, this study aimed to evaluate the cytotoxic effects of PEN, OFN, FPB, and PFE on human normal liver (THLE2) cells through investigating their impacts on cell viability, apoptosis, cell cycle, and oxidative stress.

**Material and Methods**

**Cytotoxicity by MTT Assay**

The MTT assay was conducted to evaluate the cytotoxic potential of the four herbicides (PEN, OFN, FPB, and PFE) on the normal human liver cell line THLE2 as previously described [33,34]. The cells were purchased from VACSERA (Egypt) and were grown in Dulbecco's Modified Eagle's medium (DMEM, GIBCO, Grand Island, NY, USA, Cat. no.11995073) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, Cat. no.10099133) at an initial number of 10000 cells per well. Cells were then treated by each herbicide with the following concentrations 0, 12.5, 25, 50, 100, 200, and 400 μM and were incubated for 24 h at 37°C. Cells were then treated with 12 μM (10 μl/well) MTT (Invitrogen, Waltham, MA, USA, Cat.no. M6494) and were re-incubated at 37°C for 4 h. Finally, we got rid of the MTT solution and added 100 μl dimethyl sulfoxide (DMSO, Sigma Aldrich, St. Louis, MO, USA, Cat. no.673439) for 0.5 h. Using absorbance measurements taken at 570 nm, the IC₅₀ was calculated using a sigmoidal curve plotted in GraphPad Prism 8 [35].

**Experimental Design**

THLE2 cells were allocated into 5 groups. In the control (Cnt) group, cells were treated with the vehicle (DMSO). In the PEN group, cells were treated with IC₅₀ PEN (60.50±3.19 μM). In the OFN group, cells were treated with IC₅₀ OFN (100.36±4.66 μM). In the FPB group, cells were treated with IC₅₀ FPB (174.90±6.54 μM), and in the PFE group, cells were treated with IC₅₀ PFE (186.72±6.82 μM). After all treatments, THLE2 cells were then incubated for one day until they reached suitable confluence for further analysis by qPCR, flow cytometry and spectrophotometer.

**Gene Expression by qPCR**

The qPCR was applied to measure the changes in the fold changes of apoptosis-related genes (Bax, caspase3, and Bcl2) and cell cycle-associated genes.
were incubated for one day before the addition of 10 μM DCF-DA for 1 h. The fluorescence was measured (at 485 nm for excitation and 535 nm for emission) using a fluorescent microplate reader. The level of lipid peroxide malondialdehyde (MDA) marker and the activities of the antioxidant enzymes [superoxide dismutase activity (SOD) and catalase (CAT), and glutathione peroxidase (GPx)] were measured in THLE2 cells using commercially available kits (Biodiagnostics, Cairo, Egypt) and as previously described [44–47]. Determining the amount of thioarbituric acid reactive substances (TBARS) is necessary for measuring MDA. TBA combined with MDA in an acidic media forming TBARS. SOD inhibits epinephrine's conversion to adrenochrome at a pH of 10.2, making measuring epinephrine concentrations possible. The CAT activity was determined by calculating the H2O2 dissociation rate at 240 nm.

**Statistical Analysis**

Data was analyzed statistically using GraphPad Prism 8.0. (San Diego, CA). One-way analysis of variance (ANOVA) and Tukey’s post hoc test were used to identify significant differences between groups. The data was shown as a mean ± standard error of mean (SEM), and a value of P<0.05 was used to indicate statistical significance.

**Results**

**Effect of Herbicides on THLE2 Cell Viability**

The cytotoxic potential of the four herbicides on the human normal liver THLE2 cells was determined by the MTT assay (Fig. 1). PEN, OFN, FPB, and PFE exerted cytotoxic effects on THLE2 with IC50 values of 60.50±3.19, 100.36±6.66, 174.90±6.54, and 186.72±6.82 μM, respectively. This infers that the greater IC50 values for FPB, and PFE resulted in less cytotoxicity being generated in THLE2 cells. In contrast, the greater IC50 values for PEN and OFN were much lower, leading to greater THLE2 cytotoxicity.

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**TABLE 1. Primer used for real-time PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’–3’)</th>
<th>Reverse primer (5’–3’)</th>
</tr>
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<tbody>
<tr>
<td>Bax</td>
<td>TGCTTCAGGGTTTCTACCCAG</td>
<td>GGCGGCAATCATCCTCCTG</td>
</tr>
<tr>
<td>Caspase3</td>
<td>GAAAGCGAATCACTGGACTCTGGG</td>
<td>GACCGAGATGTCATTCCAGTG</td>
</tr>
<tr>
<td>Be2</td>
<td>AGGAATGGAATTTCTGGTGCG</td>
<td>GCTAGTTCCAGGACCAGGC</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>GCTGCTCTGGTGAAACAG</td>
<td>ACAGAGGCGAGCAAGGT</td>
</tr>
<tr>
<td>CDK4</td>
<td>CATCACAGCTGGTCAGTGGG</td>
<td>TCAGTTCCGAGATGGCAGAGA</td>
</tr>
<tr>
<td>Cyclin A2</td>
<td>TGGAAAGCCAAAACGATAACAGCC</td>
<td>GGGCATTTCCAGCTTATTT</td>
</tr>
<tr>
<td>Nrf2</td>
<td>CAGCGAGCGGAAAGGATG</td>
<td>TGGCGAACCCTGGAGTAG</td>
</tr>
<tr>
<td>HO-1</td>
<td>CGGCCAGCAGAAACAGGT</td>
<td>AGTGAAGGACCCCATCGGAGAA</td>
</tr>
<tr>
<td>B-actin</td>
<td>CACCAACTGGGACGACAT</td>
<td>ACAGCTCGGATAGCAG</td>
</tr>
</tbody>
</table>

[cyclin D1, cyclin A2, and cyclin-dependent kinase 4 (CDK4)] in THLE2 cells. We first extracted total RNA from THLE2 cells using Trizol (Invitrogen, Waltham, MA, USA, Cat# 15596026). We then used RevertAid H Minus Reverse (Thermo Scientific, #E04051) to convert RNA into cDNA which was then quantified using a Nanodrop (Q5000, Quawell, San Jose, CA, USA). The qPCR test and data analysis were conducted using a Piko qPCR thermal cycler (Thermo Scientific) and its corresponding software. The PCR mixture included cDNA, primers (Table 1), and available kits (Biodiagnostics, Cairo, Egypt). We maintained the temperature range as specified by the manufacturer and as previously detailed [36,37]. Gene expression in the form of fold change was quantified using the 2−ΔΔCt method against the internal control B-actin [38,39].

**Cell Cycle by Flow Cytometry**

Flow cytometry was used to quantify the number of THLE2 in each phase of the cell cycle as previously reported [40,41]. One day after trypsinization, THLE2 cells were fixed at -20°C in 70% ethanol, underwent 3 centrifugations (10,000 rpm/5 min) with interval twice washes with 50% ethanol/PBS, and stained with 100 µl propidium iodide (PI) in PBS (640932, Biolegend, CA) for 30 min. Cells stained positive for PI were counted using flow cytometry (FACScan, BD Biosciences, USA), and the percentage of cells in each cell cycle phase was evaluated using FlowJo V.10 (BD Biosciences).

**Oxidant and Antioxidant Status**

By measuring the production of the fluorescent metabolite dichlorofluorescein (DCF) from the non-fluorescent probe 2,7-dichlorofluorescin diacetate (DCF-DA), we were able to undertake a fluorometric analysis of intracellular reactive oxygen species (ROS) as previously described [42,43]. THLE2 cells (86% confluence) were treated with each of the herbicides at IC50 value and...
Fig. 1. Cytotoxic effects of PEN, OFN, FPB, and PFE herbicides on the human normal liver THLE2 cells as detected by the MTT assay. The representative sigmoidal curves exhibited the IC\textsubscript{50} values of each herbicide as displayed within the rectangles. The values shown are the averages of three separate studies (n = 3).

The Four Herbicides Triggered Apoptosis in THLE2 Cells

To check whether the four herbicides could inhibit THLE2 proliferation through apoptosis, we conducted qPCR to evaluate the relative expression of the apoptotic genes (Bax and Caspase3) and the anti-apoptotic Bcl2 gene following treatment with the herbicides. As shown in Fig. 2, the four herbicides induced apoptosis in THLE2 cells as evidenced by significant (P<0.05) upregulation of Bax and caspase 3 and significant (P<0.05) downregulation of Bcl2 in cells treated with the herbicides compared to the control. Comparing the apoptotic effects of the four herbicides, PFE showed the lowest apoptosis followed by FPB, while PEN exhibited the highest apoptosis. These results agree with the results of the MTT assay and both assays confirmed that the four herbicides inhibited THLE2 proliferation through induction of apoptosis with lowest cytotoxic effect for PFE followed by FPB.

Effect of the Herbicides on the THLE2 Cell Cycle

To determine whether any of the four herbicides would affect the number of THLE2 in each phase of the cell cycle, PI staining was executed after treatments with the IC\textsubscript{50} value of each herbicide. As displayed in Fig. 3, the number of THLE2 cells treated with PEN significantly (P<0.05) increased in the G0/G1 phase, indicating cell cycle arrest in this phase, relative to the control cells. On the other hand, OFN induced arrest in both S and G2/M phases as revealed by a significantly (P<0.05) higher number of THLE2 cells in the two phases compared to the control group. Moreover, cells treated with PEN and OFN showed a significant (P<0.05) reduction in G2/M and G1 phases, respectively, relative to the control group. On the other hand, there were no significant differences in cell number in any phase of the cell cycle after treatment with FPB or PFE as compared to the control groups. Cell cycle arrest in different phases was further confirmed at a molecular level via the detection of relative expression of cell cycle-associated genes (cyclin D1, cyclin A2, and CDK4) by...
Effect of the Herbicides on the Oxidant and Antioxidant Status of THLE2 Cells

Treatment of THLE2 cells with OFN, FPB, or PFE led to a significant (P<0.05) increase in intracellular ROS as compared to control and PEN-treated cells, as determined by ELISA (Fig.5). In contrast, no significant (P>0.05) difference was observed in the intracellular ROS levels between PEN-treated cells and the control cells. For further confirmation, we determined MDA levels and found similar results. Among the OFN, FPB, and PFE groups, PFE exhibited the lowest ROS and MDA levels. We also detected the activities of the antioxidant enzymes (SOD, CAT, and GPx) and the expression of antioxidant-related genes (Nrf2 and HO-1) and found significantly (P<0.05) lowered levels of these antioxidant markers in THLE2 cells treated with OFN, FPB, or PFE, with lowest levels in OFN-treated cells, as compared to the control and PEN-treated cells (Fig.5). On the other hand, these antioxidant indicators were not significantly (P>0.05) different in PEN-treated cells compared to controls.

Discussion

The use of herbicides to protect crops and household plants has been an integral component of farming and gardening for decades. The global rise in the usage of pesticides that has resulted from the creation of pesticide-resistant crops is a major contributor to environmental pollution. [48,49]. Despite pesticides’ many uses, they pose a threat to human health since they bioaccumulate in vegetation, soil, water, and even the air. The use of pesticides has been linked to the onset of potentially fatal illnesses. Farmworkers’ exposure to pesticides causes DNA degradation and immunological alterations [50].

PEN treatment resulted in the maximum cytotoxicity, indicating that this herbicide may pose a threat to human cells. In agreement, several other studies reported cytotoxic potential for PEN in normal human and rodent cells. PEN induced cytotoxic effects on human umbilical vein endothelial cells (HUVECs) [17,18], Chinese hamster ovary (CHO) cells [11,12], rat hepatocytes and thyroid FRTL 5 cells [13,14], and mouse bone marrow cells [15]. The IC₅₀ of PEN (60.50±3.19 μM) on THLE2 cells was quite similar to that reported by Lee, et al. [18] on HUVECs (nearly 50 μM) after 24 h incubation. However, Saquib, et al. [17] found a very high IC₅₀ for PEN on HUVECs (500 - 1000 μM) after 4 h incubation. Another very high IC₅₀ (1-10 mM) for PEN was recorded on CHO cells following 4 h incubation [11]. Therefore, PEN could induce cytotoxicity but at different concentrations based on the type of the cells and the incubation time. OFN and FPB also induced cytotoxic effects on THLE2 cells but at IC₅₀ higher than that of PEN. In consistency, OFN inhibited cell viability in fish and other aquatic invertebrates [22-26] and FPB triggers a cytotoxic effect on mammalian cells [31]. On the other hand, to the best of our knowledge, no available study in the literature addressed a cytotoxic potential for PFE on mammalian cells, so far. Hence, our study could be the first to detect the cytotoxic effect of this herbicide on the THLE2 cells.

Real-time PCR results revealed induction of THLE2 apoptosis by the four herbicides as evidenced by higher expression of the apoptotic genes (Bax and Caspase3) and lower expression of the anti-apoptotic Bcl2 gene. These results of the MTT assay and qPCR confirmed that the four herbicides inhibited THLE2 proliferation through induction of apoptosis with lowest cytotoxic effect for PFE followed by FPB. Consistent with our findings, a prior investigation utilizing a comparable dosage of OFN also observed elevated expression of apoptotic genes (Bax, caspase 8, and caspase 9) in bovine mammary epithelial cells [28]. Similarly, other studies reported apoptotic potential for PEN on HUVECs (at a higher dose, 500 and 1000 μM) [17], and mouse testicular cells (at a lower dose 20 μM) [51] as revealed by cell cycle arrest in the sub-G0/G1 phase. Unlike FPB or PFE which did not affect cellular distribution, both PEN and OFN induced cell cycle arrest in the G0/G1 phase (via PEN) and both S and G2/M phases (via OFN) in human normal liver THLE2 cells. These data were further confirmed by qPCR which revealed a significant downregulated expression of the G1 phase-associated genes (cyclin D1 and its partner CDK4) in cells treated with PEN and a significant lowered expression of S+G2/M phases-associated cyclin A2 gene in cells treated with OFN. Consistent with our data, a previous study also reported G1 arrest and downregulated expression of cyclin D1, cyclin D3, and CDK4 in HUVECs treated with 50 and 100 μM PEN [18]. In contrast, Ansari, et al. [52] found an arrest in both...
sub-G0/G1 and G2/M phases in human lymphocytes treated with similar doses of PEN. On the other hand, our results are in agreement with Jang, et al. [28] who reported cell cycle arrest in the S, and G2/M phases with lower expression of CDK2, a marker for S/G2/M transition, in bovine mammary epithelial cells treated with OFN.

Fig. 2. Analysis of qPCR data of apoptosis-related genes (Bax, caspase3, and Bcl2) in the human normal liver THLE2 cells. Columns and error bars represented fold change mean ± SEM (n = 5/group) and the different letters [a (highest) – e (lowest)] indicate significant difference between groups at P < 0.05. Each group was measured against every other group. Cnt, control (DMSO-treated) cells, PEN, pendimethalin-treated cells, OFN, oxyfluorfen- treated cells, FPB, fluazifop-p-butyl-treated cells, and PFE, pyraflufen ethyl-treated cells.
Fig. 3. Flow cytometry histograms and representative graphs showing the impact of the four herbicides on the number of THLE2 cells in the G0/G1, S, and G2/M phases of the cell cycle. The cell count in each phase was expressed as %. Columns and error bars represented fold change mean ± SEM (n = 5/group) and the different letters [a (highest) – e (lowest)] indicate significant difference between groups at P < 0.05. Each group was measured against every other group. Cnt, control (DMSO-treated) cells, PEN, pendimethalin-treated cells, OFN, oxyfluorfen-treated cells, FPB, fluazifop-p-butyl-treated cells, and PFE, pyraflufen ethyl-treated cells.
Fig.4. Analysis of qPCR data showing the effect of the four herbicides on the relative expression of cell cycle associated genes (cyclin D1, CDK4, and cyclin A2) in the human normal liver THLE2 cells. Columns and error bars represented fold change mean ± SEM (n = 5/group) and the different letters [a (highest) – e (lowest)] indicate significant difference between groups at P < 0.05. Each group was measured against every other group.
We and Jang, et al. [28] also found a decreased number of cells in the G0/G1. In contrast, treatment with FPB or PFE did not alter cell distribution, indicating that cell cycle arrest is not required for the cytotoxic potential of these herbicides.

In the present study, THLE2 cells treated with OFN, FPB, or PFE showed higher oxidative stress (as indicated by high levels of intracellular ROS and MDA, lower levels of the antioxidant enzyme activities (SOD, CAT, and GPx), and down regulated expression of Nrf2 and HO-1 genes as compared to control and PEN-treated cells. In contrast, these oxidant/antioxidant indicators were not significantly different in PEN-treated cells compared to controls. In agreement, OFN, FPB, and PFE kill weeds through the overproduction of ROS which causes lipid peroxidation and damage to the cell membrane and finally cell death [20,29,30]. Similar oxidative stress dependant cytotoxic effects were also reported in fish for OFN [22-27], and in rat liver, kidney and testis for FPB [32]. However, no cytotoxic potential has been reported for PFE on animals or humans so far [29]. Thus, to the best of our knowledge, this could be the first study to report the cytotoxic effect of PFE on human normal liver THLE2 cells. However, compared to other herbicides used in the present study, PFE has the lowest cytotoxic effects.

Our findings highlighted the cytotoxic mechanism of four regularly used herbicides (PEN, ORF, FPB, and PFE) by assessing their effects on the survival of human normal liver (THLE2) cells as a non-target organism. Among these herbicides, PFE had the lowest cytotoxic effects against the human normal liver THLE2 cells and therefore could be used to control weeds with less adverse consequences on human health. The lack of
investigation of the cytotoxic effects of the four herbicides on another normal cell line and in vivo and their related signaling pathways is a significant shortcoming of our work. Another limitation of this study is the doses of four different herbicides employed in THLE2 that did not accurately represent actual environmental conditions. The cytotoxic effects of these herbicides on the occupational populations of workers and farmers have to be assessed as well. To properly assess the underlying mechanisms of these herbicides' cytotoxicity, further research is needed on animals and humans.

Conclusions

To the best of our knowledge, this is the first study that reported the cytotoxic potential of four commonly used herbicides (PEN, ORF, FPB, and PFE) on the human normal liver THLE2 cells with little toxicity for PFE followed by FPB. All four herbicides promoted THLE2 apoptosis, although they did so in different ways: ORF, FPB, and PFE increased ROS levels, whereas PEN and ORF caused cell cycle arrest. Although these findings provide insight into a potential threat to human health, it will be important to test these herbicides in vivo on non-targeted species at ecologically relevant amounts in the future.

Conflicts of interest

“There are no conflicts to declare”.

Funding statement

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Author’s contributions

“Authors contribute equally in this work”

References


تقييم سمية أربعة مبيدات أعشاب على خلايا الكبد الطبيعية THLE2

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الغرض من هذا البحث هو دراسة التأثيرات السمية لمبيدات الأعشاب بنديميثالين (PEN) وأوكسيفلورفين (OFN) وفلوازيفوب-بوتيل (FPB) وبيرافلوفين إيثيل (PFE) على خلايا الكبد الطبيعية للإنسان (THLE2) من خلال تقييم حيوية الخلايا والموت الخلوي المبرمج ودورة الخلية وحالة التوتر الناكتسيدي باستخدام التحاليل الكيميائية والتفاعل البيولوجي للخلايا. أدت المبيدات الأربعة إلى تأثيرات سمية على خلايا THLE2 كما يتضح من تغييرات في جينات Bax و caspase 3، وانخفاض تعبير جين Bcl2. و كلا المبيدات أدت إلى توقف دورة الخلية في مرحلة G0 / G1. ومع ذلك، فقط OFN و PFE أدت إلى توقف دورة الخلية في مرحلة G2 / M. ومن بين الميوتينات الأربعة، فقط PFE و FPB و OFN أدت إلى توقف التطور الناكتسيدي كما يظهر من مستوى كينات CDK4 و CAT و SOD و MDA و ROS. وانتظام مثابرة لإنزيمات مضاد التأكسدي للإنسان. ومن خصائص المبيدات أظهرت PFE و FPB أنماط أعلى من ROS و MDA و CAT، وأنها فعاله في تقليل التوتر الناكتسيدي من خلال استخدام في إنزيمات مضادة للإكسدة. و PFE و FPB كانت لهما دوراً في تقليل التوتر الناكتسيدي من خلال استخدام في إنزيمات مضادة للإكسدة. و PFE و FPB كانت لهما دوراً في تقليل التوتر الناكتسيدي من خلال استخدام في إنزيمات مضادة للإكسدة. و PFE و FPB كانت لهما دوراً في تقليل التوتر الناكتسيدي من خلال استخدام في إنزيمات مضادة للإكسدة.

الكلمات الدالة: بنديميثالين (PEN)، أوكسيفلورفين (OFN)، بيرافلوفين إيثيل، السمية الخلوية.