### SCREENING THE SUSCEPTIBILITY OF SOME PHOTOSENSITIZERS AS A PHOTOINSSECTICIDE ON *Spodoptera furgipedra* SF9 CELL LINE *IN VITRO*

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### ABSTRACT

The *Spodoptera furgipedra* SF9 cell line was tested by B-Phenylpyruvic acid, Hematoporphyrin IX, L-tryptophan, Mandelic acid, Histidine and L-b-phenyllactic acid for comparing the photodynamic effect. After irradiation of the tested cells, the photodynamic effect was measured with MTT assay.

The results indicated that the B-phenylpyruvic acid and Hematoporphyrin IX were the most effective photoinsecticides than the others. The L-tryptophan, Mandelic acid, Histidine and L-b-phenyllactic acids were found to have a weak photodynamic effect. So we can conclude that both B-phenylpyruvic acid and Hematoporphyrin IX were more acceptable to be used as a photoinsecticide against *S. furgipedra*.

**INTRODUCTION**

Some authors have reported the usefulness of hematoporphyrin as a photoinsecticides, Heitz (1987), Yoho, *et al.* (1976), Lenke, *et al.* (1987), Ben Amor, *et al.* (1998) and Salama, *et al.* (1998). The use of Hematoporphyrin in insect control has several advantageous features as compared with conventional insecticides. This photoinsecticide can be directly administered in aqueous solution and in association with attractive; its photophysical, photosensitizing properties have been determined in a variety of media and have been shown to be particularly efficient, Jori (1985). The primary physical process of photosensitization, the excitation of an electron, does not result in changing the spin of the electron, which is transferred to higher orbital. Therefore, the excited molecule generated is in the singlet state. Lifetimes of excited singlet states are usually very short-measured in Pico-or nanoseconds, and therefore there is a chance of chemical reactions with other molecules (substrates) as reported by Halliwell *et al.* (1993). The relatively high water solubility of hematoporphyrin, the ascertained lack of photomutagenic activity, Jori and Spikes (1983) and its wide spread clinical use as a phototherapeutic agent against solid tumours and other diseases, Jori (1986) and Brown (1997), although some potential hazards and limitation need to be considered, Heitz (1987), Yoho, *et al.* (1976), Lenke, *et al.* (1987). Photoinsecticide is also very rapidly photobleached upon exposure to UV or visible light, Jori and Spikes (1983).

It is very important to understand which biological compound is effective as a photosensitizer to be used as a photoinsecticide, in order to put the bases for the new generation of the photoinsecticide.

# In this work we throw some light on the effectiveness of the above-mentioned photosinsitizers to be used as a photoinsecticide.

The survivability of the *S furgipedra* SF9 cell line due to B-Phenylpyruvic acid, Hematoporphyrin IX, L-tryptophan, Mandelic acid, Histidine and L-b-phenyl lactic acid was determined by MTT (Tetrazolium day) assay. MTT was a colorimetric assay for anchorage depended cells, and was based on the permeability that MTT was taken up by viable cells and was reduced by the enzyme mitochondria dehydrogenase to yield purple formazan product, which was impermeable to cell membranes, resulting in its accumulation within viable /healthy cells. Solubilization of the cells by using Dimethylsulphoxide results in the release of the compound allowing its detection using a spectrophotometer.

MATERIALS AND METHODS

Chemicals

All chemicals of analytical grade were obtained from Sigma, Aldrich and Fluka (England). B-Phenylpyruvic acid was used. Stock solutions of B-Phenylpyruvic acid were prepared by dissolving a known amount of it in the minimal amount of ethanol. All different concentrations were made using PBS (Phosphate Buffered Saline) for dilution. Stock solutions of Hematoporphyrin were prepared by dissolving a known amount of Hematoporphyrin in the minimal amount of 0.1M Na OH and then neutralizing by drops of 36% HCL. The Hematoporphyrin concentration in the final solution was determined by absorption spectrophotometry, using ε =423000 M-1 cm-1 at 401.5nm. The solutions of Hematoporphyrin were stable for 4 weeks when kept in the dark at 4oC. Mandelic acid was used. Stock solutions of, Mandelic acid was prepared by dissolving a known amount of it in the minimal amount of phosphate buffer. All different concentrations were made using PBS (Phosphate Buffered Saline) for dilution. L-tryptophan, Histidine and L-b-phenyl lactic acid were also used. Stock solutions were prepared by dissolving a known amount of each of them in distilled water. Preparation of the different concentrations were made using PBS (Phosphate Buffered Saline). All the above solutions were filter sterilized before use under hood.

# **Experimental models**

*S.furgipedra* SF9 cell line was obtained from **Invitrogen**. This line was maintained for growth and subculture. This cell line originated from the IPLBSF-21cell line, derived from the pupal ovarian tissue of the fall army worm *S. furgipedra* (O`Reilly, *et al*., (1992), Vaughn, *et al*. (1977). These cells received as the following:

107 cells in 1ml 60%Grace`s medium, 30% FBS (fetal bovine serum), and 10% DMSO (Dimethylsulfoxide).

*S. furgipedra* SF9 cell line was maintained as an attached cell line at 270C in Grace’s medium [55ml of FBS (fetal bovine serum) and 500 µl of a 10 mg/ml stock of gentamycin were added to 500 ml bottles of Grace’s medium], this medium is stable for 3 months at +40C].

To determine the survivability of the *S. furgipedra* SF9 cells in presence or absence (control) of the tested photosinsitizers under UV or without UV (control), the following procedures were adopted:

1- The cells to be trypsinised were washed 2x with 5ml of PBS (Phosphate Buffered Saline) and 4ml of trypsin (1x) were added per T-75 flask. The cells were then returned to 27oC and 5% CO2 for five minutes, in order to allow cell detachment.

2- After this time, 8ml of DMEM (Grace’s medium [55ml of FBS (fetal bovine serum) and 500 µl of a 10 mg/ml stock of gentamycin were added to 500 ml bottles of Grace’s medium]) was added to neutralise the action of the trypsin, and the entire volume of fluid was removed and transferred to a sterile centrifuge tube.

3- The cells were pelleted by spinning at 1200 rpm for 5 minutes. The supernatant was removed and the cells were resuspended in fresh media.100μl of this cell suspension was removed for cell counting using trypan blue staining. 200 cells were counted by using Hemacytometer and the number of cells per ml was calculated. The dilution ratio per well was calculated, using the following ratios:

24-well plate, 5x104 cells/well

48-well plate, 2. 5x104 cells/well

1. The cells were thus seeded and media to a final volume of 1ml for 24-well plate, 0.5ml for 48-well plate was added. The plates were then transferred to 27oC and allowed to attach and grow overnight. Then the media were removed. 100μl of PBS were put per each well for washing with care. The different concentrations of the tested photosinsitizer were made from the prepared stock using PBS for dilution, 1ml was added to each 24- well plate, while 0.5 ml were added to each 48-well. Before irradiation, cells were incubated with the tested photosinsitizers for 1 hour at 27oC

The UV lamp was turned on 10 minutes prior to irradiation, in order to allow it to equilibrate. Irradiation was carried out with the cells on ice (using an ice pack wrapped in tissue), and the cells were irradiated for 2o minutes. Silver foil was placed over the control wells.

At the end of irradiation the plastic cover was replaced and the cells were put under hood .The tested photosinsitizers and PBS were removed then washed with PBS. Immediate analysis was made immediately using either trypan blue cell counts or MTT assay.

**Note**: The percentage of cells mortality was plotted against the tested concentrations and the LC50 value were determined. Mortality percentage was always corrected by Abbott’s formula (1925) if the mortality in control exceeds 5%.

##### MTT Assay

##### The (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) Thiazolyl blue was used. According to Mosmann (1983) and Yu Da, Yu Lin-Lin (1993). The *S. furgipedra* SF9 cells (250.000/0.5ml) after treatment were subjected to the following:

1. A 5mg /ml stock solution of MTT was freshly prepared in PBS, and filter-sterilized.

2- 50μl of the stock solution was added to 0.5 ml of media (48-well plate) and the cells were incubated for 1.5 hour at 27oC.MTT was to be added to samples at a ratio of 10% of the volume of the sample.

3- After the incubation, the media was removed and the cells were washed with 100μl of PBS then can be viewed under the inverted microscope.1ml of DMSO was then added to the wells and the samples were agitated gently, and the absorbance of each well was read at 570nm immediately.

### Irradiation

#### The artificial light source used was a Philips UVA lamp (HP3148/A, half body, 8TL09 lamps of 40W each, from Philips Electronics, Croydon) with an average output of 6.8x105W cm-2 UVA and 6.1x103 Wcm-2UVB. The light intensity of the lamp was measured using a Glen Spectral Radiometer, model 1680B. The irradiation dose used was 7783 Jm-2.

**The statistical analysis**

The statistical analysis was performed using t-test and analysis of variance.

**RESULTS**

Results of the present experiments are shown in Tables (1 and 2) and graphically illustrated in Figures (1,2 and3). The obtained data indicated that the cells survivability was highly affected by B-Phenylpyruvic acid and Hematoporphyrin IX as in Table (1). However, the photodynamic effect of the L-tryptophan, Mandelic acid, Histidine and L-b-phenyl lactic acid as determined by MTT assay are found to be weak in comparison with control experiments as indicated in Table (2). The response is dose-dependent, i.e. the cells mortality increases with the increase of the concentration as indicated in Table (1). The obtained data indicate also the following:

1-There is a significant increase in cells mortality due the effect of the B-Phenylpyruvic acid and the Hematoporphyrin as determined by MTT assay, (p<0.05). There was no much significance difference between the cells treated with L-tryptophan, Mandelic acid, Histidine or L-b-phenyl lactic acid as determined by MTT assay. The degree of cell inhibitions does not yield sufficient mortality to calculate LC50 ,even with higher doses.

2-The cell viability was greatly affected by Hematoporphyrin IX followed by B-Phenylpyruvic acid based on LC50 `s in Table (1) and shown graphically in Figure (3).

3-The effect of L-tryptophan, Mandelic acid, Histidine or L-b-phenyl lactic acid concentrations within the range 7.5-.001 mM/ml does not affected the cell viability in significance level to induce cells apoptosis. This was confirmed also by trypan blue staining.

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Table (1):

Survivability of the *S. furgipedra* SF9 cells (250.000/0.5ml) due to the effect of the photosensitizer B-Phenylpyruvic acid or Hematoporphyrin IX with different concentrations after UV exposure (380- 400 W/M2 artificial lights).

|  |  |  |  |
| --- | --- | --- | --- |
| Concentrations  (mM/ml) | Control without irradiation  \*Mean ± S.D. | Photosensitizer  Hematoporphyrin IX  Mean ± S.D. | Photosensitizer  B-Phenylpyruvic acid  Mean ± S.D. |
| 5 | 55.3± 1.3 7 | 0.4 ± 0.55 | 0.7 ± 0.43 |
| 2.5 | 64.87 ± 1.31 | 0.8 ± 0.97 | 1.5 ± 0.67 |
| 1 | 88.33 ± 2.70 | 15.99 ± 2.66 | 20.87± 2.43 |
| 0.25 | 92.65 ±2.88 | 57.67 ± 2.33 | 68.43 ±3.98 |
| 0.1 | 98.74 ± 0.57 | 80.47 ± 1.67 | 84.65 ±1.75 |
| 0.01 | 99.3 ± 0.7 | 90.11 ± 1.47 | 91.86 ± 1.67 |
| 0.001 | 100 | 100 | 100 |
| Lc50 |  | .15 mM/ml | .35 mM/ml |

\* The mean is the average of cells survivability of 16 well.

Table (2):

Survivability of the *S. furgipedra* SF9 cells (250.000/0.5ml) due to the effect of the photosensitizer L-tryptophan, Mandelic acid, Histidine or L-b-phenyl lactic acid with different concentrations after UV exposure (380- 400 W/M2 artificial lights).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Concentratios  (mM/ml) | Control without irradiation  \*Mean±S.D. | Photosensitizer  L-tryptophan  Mean ± S.D. | | Photosensitizer Mandelic acid  Mean ± S.D. | Photosensitizer Histidine  Mean ± S.D. | Photosensitizer  L-b-phenyl lactic acid  Mean ± S.D. |
| 7.5 | 45.89±2.56 | 40.89±3.95 | 43.97±3.11 | | 39.64±4.54 | 40.99±3.97 |
| 5 | 55.6 ± 2.7 | 50.12 ±2.55 | 47.95±4.32 | | 50.89±3.65 | 39.56 ±4.43 |
| 2.5 | 65.34±1.43 | 55.87 ±3.97 | 50.86±4.54 | | 49.75±5.64 | 44.75 ±3.67 |
| 1 | 90.56±1.70 | 60.65 ± 4.66 | 55.85±5.86 | | 59.65±3.75 | 45.4±6.43 |
| .25 | 95.54±3.88 | 85.85 ± 5.33 | 79.86±6.44 | | 88.32±4.98 | 87.95 ± 7.98 |
| .1 | 98.85±1.57 | 90.94 ± 5.67 | 93.87±4.53 | | 91.87±4.95 | 90.65 ±6.75 |
| .01 | 99.4 ± 0.6 | 99.1 ± 0.47 | 95.3±0.21 | | 96.32±.64 | 92.65 ± 0.67 |
| .001 | 100 | 100 | 100 | | 100 | 100 |

\*The mean is the average of cells survivability of 16 well.

DISCUSSION

The use of cell cultures for studying the effect of different insecticides instead of intact larvae provides several advantages, including reduced time, cost for experimentation, and absence of obscuring enzyme systems typically present in insects and a homogenous cell population lowering the strict measurement of insecticides effects. The cytotoxicity testes indicated that the *S. furgipedra* SF9 cell line is quit susceptible to the photodynamic effect of both Hematoporphyrin IX and B-phenylpyruvic acid as measured by MTT assay. The reactive oxygen is greatly affected the cells as the concentration increases resulted in cells apoptosis (cell death).

The results indicated that the use of *S. furgipedra* SF9 cell line is a good model for studying the susceptibility of this insect cell to the tested Photosensitizer. This finding agrees well with, Hasain *et al.* (1999) who reported that the cellular imbalance in the levels of antioxidants and reactive oxygen species (ROS) is directly associated with a number of pathological states and results in programmed cell death or apoptosis. They demonstrated that the use of in vitro cultured *S. furgipedra* SF9 insect cells as a model to study oxidative stress induced programmed cell death. They added that apoptosis of in vitro cultured SF9 cells were induced by the exogenous treatment of H2O2 to cells growing in culture. The AD50 (concentration of H2O2 inducing about 50% apoptotic response) varied with the duration of treatment, batch-to-batch variation of H2O2 and the physiological state of cells. Also Kwa, *et a.l* (1998) concluded that the *S. furgipedra* SF9 insect cell line was most sensitive cell line towards the effect of the *Bacillus thuringiensis* delta-endotoxin Cry1C to cultured insect by means of toxicity assays.

The present results indicated that Hematoporphyrin IX and B-Phenylpyruvic acid were very highly toxic to the tested *S. furgipedra* SF9 cell line as a photoinsecticide. These results confirm the findings of Jori and Spikes (1983). They concluded that the rapid insecticidal action of Hematoporphyrin could be correlated with its mode of photo inducing irreversible damage to biological systems. Our results agree will with, Ben Amor *et al*. (1998). They pointed out that Porphyrins may represent a class of useful photoinsecticidal agents in particular, Hematoporphyrin appeared to be very active against at least two fly species, namely *Ceratitis capitata* and *Bactrocera oleae* which are known to induce severe damage in various agricultural areas worldwide.

The results indicated that the photoinsecticidal activity depends on the dose used as indicated by the LC50 `s of Hematoporphyrin IX and B-Phenylpyruvic acid (.15 mM/ml &. 35 mM/ml) respectively. However, our results indicated also that the L-tryptophan, Mandelic acid, Histidine and L-b-phenyllactic acid have weak photodynamic effect in comparison with Hematoporphyrin IX and B-Phenylpyruvic acid, even with high concentrations. Our results confirmed this phenomenon because on increasing the concentration the solution become more turbid restricting the UV light to act well in inducing the photodynamic effect to the treated cells. This finding was found to be in agreement with He Liming, et al (1997), in their test for comparing the photodynamic effect of 49 single laser photosensitizers on the SW1116 cell line of human colorectal cancer and K562 cell line of erythroleukemiwith MTT assay. They concluded the effectiveness of 16 single Photosensitizer from 49 one to be used in photodynamic killing of human cancer cell lines in *vitro*.

The results confirmed the efficiency of both B-Phenylpyruvic acid and Hematoporphyrin IX as a photoinsecticide against *S furgipedra*. Thus it may recommend the usage of the tested toxicants in control management strategy to reduce costs and pesticide impact in the environments.

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