The In-Vitro Toxic Effect of The Glycoalkaloids for Some Solanum Species Against The LIM-1863 Cell Line

Ahmad Sammani¹, Esam Shammaa², Fawaz Chehna² and Abdulkader Rahmo³

¹Pharmacognosy Department, Faculty of Pharmacy, University of Damascus, Damascus, Syria
²Pharmaceutical Chemistry Department, Faculty of Pharmacy, University of Aleppo, Aleppo, Syria
³National Commission for Biotechnology, Damascus, Syria

ABSTRACT

Background The LIM-1863 Cell Line is one of the colon cancer types considered to be responsible for a high rate of deaths, and the glycoalkaloids being natural substances existing in the Solanum species have anticancer effects.

Objective This research aims at studying the effect of the glycoalkaloids on viability of the LIM-1863 cancerous cells in-vitro.

Materials and Methods The glycoalkaloids in this study are extracted by the ultrasonic waves technique and detecting them by the Thin Layer Chromatography (TLC) in addition to incubating of the LIM-1863 cells with different concentrations of the glycoalkaloids for 48 hours and then assessing of the cell viability using the MTT assay.

Results The findings showed that the glycoalkaloids have a toxic effect on the LIM-1863 cells and that half of the inhibiting concentration (IC₅₀) of the Solanum fruits extract: (Solanum nigrum L.), (Solanum villosum Mill.) and (Solanum elaeagnifolium Cav.) on the LIM-1863 cells have the (164.7, 35.91 and 12.14 µg/ml) values successively.

Conclusion The observations indicated that the glycoalkaloids are able to inhibit the colon cancer cell proliferation.

Keywords: Solanum, Glycoalkaloids, TLC, LIM-1863, Viability, MTT.

INTRODUCTION

Historically, plants have a useful effect on many diseases that infecting the human life, as the World Health Organization estimates that about 80% of the health problems of the globe’s population should be treated by the medical plants drugs[1,2]. The medical plants have a long history for cancer treatment because the active constituents of Angelica gigas, Camptotheca acuminate, Catharanthus roseus, Ocrasia elliptica, Podophyllum emodi, Podophyllum peltatum and Taxus brevifolia have been used to treat advanced stages of several malignant tumors[3]. The colon cancer is responsible for a high rate of deaths being directly connected to age and diet that spreads more over time[4,5].

The glycoalkaloids are considered among the most important compounds with an anticancer effect which are found in the butanol’s extracts of fruits (BEF) of the Solanum species spreading in the Syrian wilderness. The (S. nigrum), (S. villosum) and (S. elaeagnifolium) (Figure 1) categorized under the Solanaceae family according to Syria’s Poisonous Plants Information System (SPPIS)[6].

The Solanum genus is regarded one of the largest genus of the Solanaceae family since it contains more than 1500 species many of which are economically important throughout their cosmopolitan distribution such as annual and perennial plants, forbs, vines, sub-shrubs, shrubs and small trees, and they often have attractive fruits and flowers. The Solanum constituents moreover have medicinal and toxic values together[7,8].

*Corresponding author:
Ahmad Sammani
New Aleppo,
Aleppo,
Syria
E-mail: abolhasanph@gmail.com

DOI: 10.5530/pj.2014.4.4

Figure 1: The profile of fruits of Solanum Species
The *Solanum* contains the glycoalkaloids that showed biological effects like the antifungal and the antiviral ones, but its most important feature in fact lies that it also shows considerable anticancer effects. For example, The Solamargine causes the human hepatoma cells death (Hep3B) by apoptosis and the antiviral ones. From *S. crinitum* and *S. jabrense* has a cytotoxic effect on the leukemia cells chaconine, solanine, tomatine, and their derivatives inhibit the human colon (HT29) and liver (HepG2) cancer cells to grow. β-2-solamargine from *Solanum nigrum* has a toxic effect on the cell lines: HT-29 (colon), HCT-15 (colon), LNCaP (prostate), PC-3 (prostate), T47D (breast), and MDA-MB-231 (breast).

This research has the aim to find the toxic effect of the glycoalkaloids existing in the BEF of *Solanum* species fruits on a type of human colon cancer being the LIM-1863 cell line, to extract the glycoalkaloids from the *Solanum* plant fruits and then to separate and detect them using the Thin Layer Chromatography (TLC) and the chemical reagents.

**MATERIALS AND METHODS**

**Plant materials**

The botanical materials that are studied in this research have been collected in September 2010 where the *S. nigrum* and the *S. villosum* fruits were collected from Ebla University’s Garden (GPRS-data: 35° 54’ 34” N and 36° 51’ 17” E) while the *S. elaeagnifolium* fruits were collected from Al-Raqqa city (GPRS-data: 35° 57’ 1” N and 39° 9’ 40” E) (Figure 3) and all documented by Dr. Amin Salkiny (ICARDA, Aleppo, Syria). The fruits are placed on stainless clean trays and stored at the room temperature for 7 days. The dry fruits are weighed, ground to become a fine powder and kept within dry containers in a fridge.

**Extraction of glycoalkaloids**

The glycoalkaloids were extracted from the *Solanum* species fruits by the ultrasound technique using the butanol as a solvent. A design of such extracting is found in (Figure 4). The obtained butanol's extract is filtered through filtering papers and concentrated using the rotary evaporator (B465, Buchi, Switzerland) to give (4.5, 2.5 and 2.5% w/w) for BEF of *S. nigrum*, *S. villosum* and *S. elaeagnifolium*, respectively. The BEF of *Solanum* species were stored in -20 °C until their use. The concentration used in the experiment based on the extract's dry weight (µg/ml).

**Analysis of glycoalkaloids by TLC**

The preliminary examination of the previously extracted glycoalkaloids using TLC (Silica Gel 60 GF254, Merck, Darmstadt, Germany). The solvent system used was chloroform: methanol: water (14:6:1), the chromatogram is sprayed in Dragendorff’s reagent, phosphomolybdic acid reagent, ninhydrin reagent 2% (then ultra-violet UV 365nm), antimony trichloride reagent 2%, and blood hemolysis reagent.

**Cell culture and glycoalkaloids treatment**

LIM-1863 cell line was obtained from Prof. Nizar Mhaidat, Jordan University of Science and Technology. The LIM-1863 cells were cultured in DMEM medium added to which 10% fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin and 1% streptomycin (All from Gibco, Scotland) at 37 °C within an incubator contains Co2 5%. The cells which were harvested by
trypsin 10% (Sigma, USA) are counted using neubauer slide and trypan blue, and they were then cultured in 96-well plate (1 × 10^4 cells/well). The BEF was dissolved in ethanol 50%. The cells were incubated with different concentrations of BEF (5, 20, 80 and 320 µg/ml) for 48 hours. Each concentration was tested on four wells of the 96-well plates which contain 1 × 10^4 LIM-1863 cells. In every experiment, four LIM-cells cultured wells without a sample are used as a negative control and four culture-medium wells without cells are used as a blank[25].

**Determination of cell viability**

The cell viability is determined using the methyl thiazoly l tetrazolium bromide (MTT) (Sigma, USA) assay[25]. The MTT was dissolved in PBS (phosphate buffer saline) at a concentration of 5 mg/ml. After the cells have been incubated for 48 hours, 20 µl of MTT solution is added to each well containing 150 ml of culture medium. The yellowish MTT solution is changed to insoluble purple formazan crystals within 24 hours of incubation (Figure 5). Presence of active mitochondrial enzyme in the living cells is the reason for this change while such enzyme is not available in the dead cells (Figure 6).

**Figure 5:** The insoluble purple formazan crystal
The purple formazan crystals are dissolved in 150 ml of DMSO (Sigma, USA) (Figure 7). Absorbency of the solution is read using a multi-well spectrophotometer (ELISA reader, Organon Teknika, Netherlands) at a wavelength of 570 nm. The percentage of viability was calculated according to following formulas:

\[
\% \text{ cell viability} = \frac{(A_t - A_b)}{(A_c - A_b)} \times 100
\]

Where, \(A_t\) = Absorbance value of test compound, \(A_b\) = mean absorbance value of blank, \(A_c\) = mean absorbance value of negative control.

Repeat the above steps, but the concentrations of (BEF) are (0.5, 6, 12,18,25,37 and 50 µg/ml) from \((S. villosum)\) and (0.25, 3, 6, 9, 12,18 and 25 µg/ml) from \((S. elaeagnifolium)\) at 48 h.

Statistical analysis

Results were expressed as mean ± SD by Excel MS Office\(^{26}\).

RESULTS

Detection of glycoalkaloids by TLC

It has been indicated through the TLC chromatogram the availability of several compounds in the butanol’s extracts of the \(Solanum\) species fruits (Figure 9), some of these compounds are alkaloids with \(R_f\) values the following: (0.07, 0.13, 0.17, 0.20, 0.27, 0.39, 0.52, 0.59, 0.63) and among such compounds there are ones with saponin properties called alkaloid steroid saponins having \(R_f\) values the following: (0.27, 0.52). It is noted here that the Dragendorff’s reagent and the blood reagent are less sensitive to the little-amount concentrations than the rest of the used reagents (Table 1).
Glycoalkaloids Against LIM-1863 Cell Line

Table 1: TLC of glycoalkaloids of three of Solanum Species

<table>
<thead>
<tr>
<th>$R_f$ ±0.01</th>
<th>Fruits</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>0.07</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.13</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.17</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.20</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.27</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.39</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.52</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.59</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.63</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.69</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.74</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- : Absent, + : Present

Glycoalkaloids cytotoxicity on LIM-1863 cells

It appeared by the preliminary experiment in comparison to the control that the Glycoalkaloids have a toxic effect on the LIM-1863 cell line (Figure 10), and the IC$_{50}$ values were like this: (164.7, 35.91 and 12.14 µg/ml) for the butanol's extracts of (S. nigrum), (S. villosum) and (S. elaeagnifolium) fruits, respectively (Figure 11). While appeared by the second experiment in comparison to the control that the IC$_{50}$ values were like this: (14.6 and 57.6 µg/ml) for the butanol's extracts of the (S. villosum) and (S. elaeagnifolium) fruits, respectively (Figure 12), being approximate to the IC$_{50}$ values in the first experiment.
**Figure 10:** The first test: the viability of LIM-1863 colon carcinoma cells after 48 hours of exposure to different concentrations of each extract, which was assessed using MTT assay. Results are expressed as a percentage of viability compared to control and are presented as mean ± SD of three independent experiments.

**Figure 11:** The IC50 values for Glycoalkaloids affecting on LIM-1863 cells during 48 h incubation. That were assessed by MTT assays. Results were expressed as mean ± SD.
DISCUSSION

It has been observed that the natural products are good and important sources to develop new drugs including anticancer drugs. But in the present study, the butanol’s extract of (*S. elaeagnifolium*) fruits has the greatest toxic effect on cell viability and then follows the butanol’s extract of (*S. villosum*) fruits. Then, the one with the least effect is the butanol’s extract of (*S. nigrum*) fruits.

In comparison with the reference studies exhibiting the *Solanum* species is a rich source of glycoalkaloids and plants of this species are in the first place contain glycoalkaloids such as solamargine, solasonine and solanine, and it appeared that solanine shows an anticancer effectiveness at (14.47 µg/ml) IC$_{50}$ value, solamargine shows an anticancer effectiveness at about (~ 4.6 µg/ml) IC$_{50}$ value and solasonine shows an anticancer effectiveness at an average value of (2.3 µg/ml) IC$_{50}$, our results have been approximate to the glycoalkaloids’ role in toxicity generating and because both butanol’s extracts of (*S. villosum*) and (*S. elaeagnifolium*) fruits have reduced the LIM-1863 cell viability at (4,12 µg/ml) IC$_{50}$ values respectively, which means they both contain a larger percentage of glycoalkaloids, while IC$_{50}$ value of (*S. nigrum*) fruits’ butanol’s extract (164.7 µg/ml) is high indicating they contain a less concentration of the glycoalkaloids (Table 2). This corresponds with the reference study.

**Table 2: Comparison of IC$_{50}$ values between results of reference studies and results of this study**

<table>
<thead>
<tr>
<th>Material and Species</th>
<th>Dry part used</th>
<th>Solvent used in extraction</th>
<th>Cell line</th>
<th>Origin</th>
<th>IC$_{50}$ µg/ml</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. nigrum</em> fruits</td>
<td>-</td>
<td>methanol</td>
<td>HELA</td>
<td>cervical</td>
<td>265</td>
<td>[27]</td>
</tr>
<tr>
<td><em>S. nigrum</em> herb</td>
<td>-</td>
<td>ethanol 70%</td>
<td>HELA</td>
<td>cervical</td>
<td>227</td>
<td>[28]</td>
</tr>
<tr>
<td>solanine</td>
<td>-</td>
<td>-</td>
<td>HepG2</td>
<td>liver</td>
<td>14.47</td>
<td>[29]</td>
</tr>
<tr>
<td>solamargine</td>
<td>-</td>
<td>-</td>
<td>H441</td>
<td>lung</td>
<td>3.5</td>
<td>[30]</td>
</tr>
<tr>
<td>solamargine</td>
<td>-</td>
<td>-</td>
<td>H520</td>
<td>lung</td>
<td>7.7</td>
<td>[30]</td>
</tr>
<tr>
<td>solamargine</td>
<td>-</td>
<td>-</td>
<td>H661</td>
<td>lung</td>
<td>8.3</td>
<td>[30]</td>
</tr>
<tr>
<td>solamargine</td>
<td>-</td>
<td>-</td>
<td>H69</td>
<td>lung</td>
<td>6.7</td>
<td>[30]</td>
</tr>
<tr>
<td>solamargine</td>
<td>-</td>
<td>-</td>
<td>HBL-100</td>
<td>breast</td>
<td>2.4</td>
<td>[31]</td>
</tr>
<tr>
<td>solamargine</td>
<td>-</td>
<td>-</td>
<td>SK-BR-3</td>
<td>breast</td>
<td>3.5</td>
<td>[31]</td>
</tr>
<tr>
<td>solamargine</td>
<td>-</td>
<td>-</td>
<td>ZR-75-1</td>
<td>breast</td>
<td>2.5</td>
<td>[31]</td>
</tr>
<tr>
<td>solamargine</td>
<td>-</td>
<td>-</td>
<td>HCT116</td>
<td>colon</td>
<td>4.6</td>
<td>[32]</td>
</tr>
<tr>
<td>solasonine</td>
<td>-</td>
<td>-</td>
<td>HCT116</td>
<td>colon</td>
<td>2.3</td>
<td>[32]</td>
</tr>
</tbody>
</table>

Figure 12: The second test: the viability of LIM-1863 colon carcinoma cells after 48 hours of exposure to different concentrations of each extract, which was assessed using MTT assay. Results are expressed as a percentage of viability compared to control and are presented as mean ± SD of three independent experiments.
which shows the IC$_{50}$ value of (S. nigrum) extract on average equals (246 µg/ml).

**CONCLUSION**

The present study shows that glycoalkaloids from *Solanum Species* clearly have the capacity to cancer cell death and these natural products represent interesting lead compounds for the development of potential cancer therapeutics. This is the first report which was tested effect (BEF) of *Solanum Species* on the viability of LIM-1863 human colon carcinoma cell line. Our data suggest that the presence of glycoalkaloids in *Solanum Species* is associated with cancer cell death.

**ACKNOWLEDGEMENTS**

The authors acknowledge all the staff members of Departments of Pharmacognosy of Faculty of Pharmacy, University of Damascus, Syria. And their thank to all the staff members of the National Commission for Biotechnology, Damascus, Syria: Maisson Elwi, Manal Saleh and Inas Nimr for their helps in culture techniques, and for the financial support provided by the National Commission for Biotechnology.

**REFERENCES**