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Cytotoxicity of *Solanum nigrum* L green fruits on breast (MCF-7) and liver (HepG-2) cancer cell lines

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Abstract

Medicinal plants have been used for decades for the treatment of different diseases. *Solanum nigrum* L is an important medicinal plant in many traditional health care systems. It has been used for the management of liver disorders, diabetes, infections, cancer, inflammations and peptic ulcer. In the present study, we screened the green unripe *S. nigrum* L fruits and leaves for their cytotoxic activity. The ethanolic extract of green fruits revealed a marked cytotoxicity against breast (MCF-7) and liver (HepG-2) cancer cell lines with IC₅₀ of 12.7 and 16.6 µg/mL, respectively. This activity is related to the high content of steroidal glycoalkaloids in *S. nigrum* L unripe green fruits.

Keywords: Medicinal plants; *Solanum nigrum* L; Cytotoxicity; Steroidal glycoalkaloids.

1. Introduction

Medicinal plants are used worldwide for the treatment and prevention of diseases. Most of the new drugs introduced to the market were developed from medicinal plants and other natural sources [1]. In developing countries, the majority of the population uses their own traditional medicine. There are more than 20,000 species of medicinal plants used in traditional medicines, and they are potential sources for new drugs [2]. *Solanum nigrum* L. (Solanaceae), commonly known as "Black nightshade," has been extensively used in traditional medicine for treatment liver problems [3], seizures [4], peptic ulcers [5], diabetes mellitus [6], chronic skin infections, inflammatory conditions, pain, fever, eye diseases and cancer [7]. *S. nigrum* L contains several bioactive metabolites such as the steroidal glycosides solamargine and solasonine that have a potent anticancer properties [8]. Also, it is a rich source of polyphenolics such as gallic acid, catechin, caffeic acid, epicatechin, rutin, and naringenin [9]. The green unripe fruits of *S. nigrum* L contain more quantity of steroidal glycoalkaloids than the ripe fruits [10].

Worldwide, hepatocellular carcinoma (HCC) is the fifth most common malignancy, its annual incidence is ranging between 3 - 9% [11]. Breast cancer is the most frequent malignant tumour in women worldwide [12]. In Egypt, it represents 20% of total cancer cases, during the year 2001 [13].

In the current study, we have investigated the in vitro cytotoxicity of the ethanolic extracts of the green unripe fruits of *S. nigrum* L, which was compared to the ethanolic extracts of the plant leaves on human breast adenocarcinoma cell line (MCF-7) and human liver hepatocellular carcinoma cell line (HepG-2), using doxorubicin as a standard anticancer agent.

2. Material and methods

2.1. Plant material

Fresh plant material of *Solanum nigrum* L was collected from Nahda University campus, East Desert, Beni-Suef, Egypt in January 2012. It was authenticated by Prof. Abd El-Halim Mohamed, Department of Flora and Phytotaxonomy Research, Horticulture Research Institute, Agriculture Research Center, Dokki, Cairo, Egypt. Fresh leaves and fruits were separated, and air dried. The powdered organs were used for phytochemical and biochemical investigations.

2.2. Preliminary phytochemical screening

Preliminary phytochemical screening of ethanolic extracts of *S. nigrum* L fruits and leaves were performed for the presence of saponins, steroids, glycosides, tannins, alkaloids, flavonoids, and carbohydrates by the chemical tests described by Khandelwal [14].

2.3. Preparation of plant extracts

Dried powdered fruits and leaves (100 g, each) were separately extracted with 95% ethanol till exhaustion, and then each of the ethanolic extracts of leaves and fruits was concentrated under reduced pressure using a rotary evaporator.

2.4. Cell lines

MCF-7 and HepG-2 cancer cell lines were kindly supplied by Pharmacology Department, Faculty of pharmacy, Al Azhar University, Egypt. The media were sterilized by 0.22 μm microbiological filters and kept at 4 °C before use.

2.5. Cytotoxicity Assay (Sulforhodamine B assay)

2.5.1. Principle

Sulforhodamine B (SRB) is a bright pink Aminoxanthine dye. Under mild acidic conditions, SRB binds to basic amino acid residues of the cellular proteins in Trichloroacetic acid fixed cells to provide an estimate of total protein mass, which is related to cell number using doxorubicin[®] as a standard [15].

2.5.2. Procedure

Different concentrations of the extracts under test (0, 0.05, 0.5, 5, 50 and 500 $\mu\text{g}/\text{mL}$) were added to the cell monolayer triplicate wells. Monolayer cells were incubated with the extracts for 48 hrs at 37 °C and in atmosphere of 5% CO_2 . After 48 hrs, cells were fixed by Trichloroacetic acid, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in a microplate ELISA reader at 492 nm, subtracting the background measurement at 620 nm. Triplicate repeats were performed for each concentration and the average was calculated.

2.5.3. Calculations

Data is expressed as the percentage of relative viability, which was calculated using the following equation:

Relative viability = (treated cells absorbance/control cells absorbance) \times 100.

The positive cytotoxic activity is indicated by less than 100% relative viability. The relation between surviving fraction and extract concentration is plotted to get the survival curve of liver, and breast cell lines. Also the IC_{50} (dose of the extract which reduces survival to 50%) for active extracts was calculated.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical study of *S. nigrum* L fruits and leaves ethanolic extracts revealed the presence of alkaloids, steroids, flavonoids, carbohydrates tannins and saponins (Table 1).

3.2. Cytotoxicity of *S. nigrum* L extracts

When screened for their cytotoxic effect (table 2) ethanolic extract of the unripe fruits exhibited marked cytotoxic activity on the breast (MCF-7) and the liver (HepG-2) cancer cell lines. However, the ethanolic extract of the unripe leaves showed much less cytotoxic activity on the same cancer cell lines.

Table 1: Preliminary phytochemical screening of the ethanolic extracts of *S. nigrum* L unripe fruits and leaves.

Test	<i>S. nigrum</i> leaves	<i>S. nigrum</i> fruits
Crystalline sublimate	-	-
Steam volatile substance	-	-
Carbohydrates &/or glycosides	++	++
Condensed tannins	-	-
Hydrolysable tannins	++	+
Flavonoids	++	+
Saponins	+	++
Sterols &/or triterpenes	++	++
Alkaloids &/or nitrogenous bases	+	++
Free anthraquinones	-	-
Combined anthraquinones	-	-
Cardiac glycosides	-	-

(+) Indicates presence of constituents and (-) indicates absence of constituents

Table 2: The evaluated IC_{50} ($\mu\text{g}/\text{mL}$) of *S. nigrum* leaves and green fruits ethanolic extracts on selected cancer cell lines.

Extract	MCF-7 ($\text{IC}_{50} \pm \text{SE}$) [*]	HepG-2 ($\text{IC}_{50} \pm \text{SE}$) [*]
<i>S. nigrum</i> leaves extract	89.1 \pm 2.57	95.7 \pm 2.88
<i>S. nigrum</i> fruits extract	12.7 \pm 3.64	16.6 \pm 3.1
Doxorubicin [®]	0.9 \pm 1.3	0.9 \pm 0.8

* $p < 0.05$

4. Discussion

In the present study *S. nigrum* L ethanolic extract of the unripe green fruits exhibited a potential cytotoxic effect on breast and liver cancer cell lines which was not the case of the unripe leaves. In previous studies, *S. nigrum* L fruits extract showed cytotoxicity against cervix, kidney [16] and colon cancer [17]. Cytotoxicity of *S. nigrum* L mainly is attributed to its steroidal glycoalkaloids content, which are present in higher concentrations in the green unripe fruits [10]. These results need further *in vivo* studies to evaluate the potential anticancer properties of *S. nigrum* L green unripe fruits.

5. Conclusion

Solanum nigrum L green fruits are considered a potential new natural remedy for breast and liver cancer that need further *in vivo* and clinical investigations to confirm its efficacy.

6. References

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