EVALUATION THE ACTIVITY OF CHLOROFORM EXTRACT OF SOLANUM NIGRUM ON NON HODGKIN LYMPHOMA CELL LINE (SR), RAT EMBRYO FIBROBLAST CELL LINE (REF) AND HUMAN LYMPHOCYTES IN VITRO

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ABSTRACT

Objective: The present study was designed to evaluate in vitro the cytotoxic activity of chloroform fruit extract (mature-black, and immature- green) of Solanum nigrum L. against Non Hodgkin Lymphoma (SR) Cell Line and Rat Embryo Fibroblast (REF) Cell Line. In addition, involves their effects on the division of the normal human lymphoid cells (Mitotic Index IM).

Methods: The fruits of S. nigrum were prepared to extract by chloroform with serial dilution in concentrations (312.5, 625, 1250, 2500, 5000, and 10000) µg/mL using MTT assay.

Results: The results show existence of clear significant (P≤0.05) cytotoxic effect of Solanum nigrum chloroform extract on SR cancer cell line. The green berries were more effective. The highest inhibition rate was 90.05% in concentration 10000µg/mL and IC₅₀ was 669.27µg/mL for exposures time 72hr. While on REF cell line, the green berries extract was the highest inhibition rate54% in concentration 10000µg/mL and IC₅₀ was 7628.1µg /mL for exposures time 72hr. The crude extracts did not have the ability to motivate the cell division of the lymphoid cells. Mitotic Index.

Conclusions: Chloroform extract of green berries show greater activity on SR cancer cell line and little activity on REF cell line. No effect on motivate or stopping human lymphocyte cells.

KEYWORDS: Solanum nigrum, Non Hodgkin Lymphoma, Rat Embryo Fibroblast.
INTRODUCTION
Natural Products have long been a fertile source of cure for cancer, which is projected to become the major causes of death in this century. However, there is a continuing need for development of new anticancer drugs, drug combinations and chemotherapy strategies, by methodical and scientific exploration of enormous pool of synthetic, biological and natural products.[1] There are various medicinal plants reported to have anti-cancer as well as anti-inflammatory activity in the Ayurvedic system of medicine.[2] Solanum nigrum (solanaceae family) one of them with proven anti-cancer as well as anti-inflammatory activity.[3, 4]

Cancer is one of the major causes of death worldwide. It was estimated 12.7 million people were diagnosed, and about 7.6 million of them died in 2008.[5] As evaluated in this report more than 2 million new cancer cases and 13 million deaths are by 2030.[6] It is well known that chemotherapy and radiotherapy are toxic not only to cancer cells, but also to healthy cells. However, the use of nature sources, particularly plant-derived products in cancer treatment and many side effects of conventional therapy reduced.[7] S. nigrum possesses various compounds that are responsible for diverse activities. The major active Components are glycoalkaloids, glycoproteins, and polysaccharides. It also contains polyphenolic compounds such as Gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin.[8] The glycoalkaloids include solamargine, solasonine. It comprises 95 percent of the total alkaloid concentration present in the plant and is found naturally in any part. It is one of the plant’s major natural defenses as it is toxic even in small quantities. With a molecular weight of 868.04 and formula C45H73NO15 it consists of an aglycone, solanidine (alkaloidal portion), and three sugar moieties (glucose, galactose, and rhamnose, collectively known as solatriose), which are attached to the third position of the aglycone.[9, 10] It is generally present in the form of α-solanine, but can be hydrolyzed to β- and γ-solanine with one or two carbohydrate molecules each.[11] These glycoalkaloids demonstrate marked antitumor effects on various tumor cell lines.[12]

MATERIALS AND METHODS
Plant Collection
The fruits of Solanum nigrum were collected from many areas of Iraq; Al-Anbar University garden, Dialla, Anah, and Alrashdiya, during the period June 2013-February 2014. The plant was identified by Professor Ali Al-Mosawy, Ph.D. in Plant Taxonomy, Department of Biology, College of Science/University of Baghdad.
Chloroform Extract

Fresh Fruits of *S. nigrum* were washed and prepared to extract at Pharmacy College /Al-Mustansiriyyah University. Which the extraction and the other Laboratory investigations were carried out.

According to,[13,14] 50 gm of fresh fruits were mixed with 50-70 mL of Chloroform. Then the sample was homogenized in electric shaker for 5 minutes. The mixture was put in thimble in souxhlet extractor and with 200 mL of chloroform for total time 8 hours. The solvent was removed by rotary evaporator under pressure at 40°C until it turned into sticky mode and kept in the refrigerator until use. Serial dilution of chloroform crude extracts (312.5, 625, 1250, 2500, 5000, and 10000) µg/mL were prepared in serum free media for cytotoxicity assay.

Chemical tests

Several chemical on the chloroform extracts of *S. nigrum* testes of general constitute were carried out; Dragendorff's Test for Alkaloids, Mayer's Test for Tannins, Foam test for Saponins, Fehling's reagent test for Glycosides, Test for Coumarins, and Resins.[13,15]

Preparation of cell lines

Cancer cell line used was Human lymphoma cell line originated in 1983 by Walter J. Urba and Dan L. Longo. Caucasian male 11 years old, it was taken from pleural effusion tissue. The morphology was lymphoblast, and the disease: large cell immunoblastic lymphoma.[16] Passages 3-12, and transformed cells of Rat Embryo Fibroblast. Passages 92-95. The normal culture of the rat embryo is the most important source for undifferentiated fibroblastic culture.[17] These were supplemented from Iraqi Center and Medical Genetic Research. These cell lines were grown and maintained using RPMI-1640 supplemented with 10% FBS.

Cytogenetic study on lymphocyte of human circulating blood

Samples collection

Blood was taken from normal adult human by puncturing using disposable syringe 5 ml of blood was transferred in to heparinized tubes. The test inhibitory effect on normal cells is an important and basic thing that must be investigated when studying the effect of substance to be used therapeutically, and most important of these cells, particularly lymphocytes, which have the ability to divide outside the body influence by division factors.
MTT Assay
Cell proliferation (viability) was evaluated by MTT assay. MTT is a yellow colored water soluble tetrazolium dye. Mitochondrial enzyme lactate dehydrogenase, produced by metabolically active cells reduces MTT to water-insoluble formazan crystals. When dissolved in appropriate solvent, these formazan crystals exhibit purple color. The intensity of the purple color is directly proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. MTT (0.5g) was dissolved in 100 mL of PBS in order to prepare a 5mg/mL concentration of the dye.\[^{18}\]

Statistical Analysis
Statistical analysis was performed using SPSS V.21 (Statistical Package for Social Science) and CompuSyn V.1. SPSS software was performed to analyze data by using two way analysis of Variance (ANOVA). Student LSD was used to assess significant difference among means (P ≤ 0.05) was considered statistically significant.

Concentrate inhibition (50%) Cl\(_{50}\) was performed by using CompuSyn.\[^{19,20}\]

RESULTS
Chloroform extracts for green berries were yield (3.420 g), about 6.84%, viscous textures, greenish-yellow color, with strong odor. Black berries were yield (1.310 g), about 2.6%, viscous textures, dark green color, with strong odor. Table (1) show results of chemical tests for chloroform extracts of \(S.nigrum\) fruits. The effects of treating SR cell line with the chloroform extracts of \(S.nigrum\) are shown in Figures (1, 2). The chloroform extract of black berries showed a time-dependent and concentrations effect on growth inhibition on SR cells. The highest inhibition was after 72h. of exposure 88.65% at concentration 10000µg/mL and the lowest was 38.36% at concentration 312.5 µg/mL for the same time of exposure. While the highest inhibition for green berries were 90.05% at concentration 10000 µg/mL and the lowest was 39.29% at 312.5 µg/mL after 72h. of exposure. It was a significant (P≤0.05) inhibitory effect in all concentrations. The effective IC\(_{50}\) values were show in table (2). The effects of treating REF cell line with the chloroform extracts of \(S.nigrum\) are shown in Figure (3). The results showed the absence of significant differences (P≤0.05). There was no clear toxic effect in concentrations except the percentage (50.97%) at the 10000 µg/mL for black berries and the percentage (54.0%) at 10000 µg/mL for green berries. While IC\(_{50}\) was 9530 µg/mL for black berries and 7628.1 µg/mL for green berries, as show in table (3).
Phytohemagglutinin (PHA) is an extract from Phaseolus vulgaris works to separate the red blood cells from cancer cells for the purpose of studying in vitro.\textsuperscript{[21]} The lymphocytes in the blood does not fall under routine conditions, because a specialized adult cells, but can be stimulated to divide mediated by a number of Alketanat stimulating mitosis as PHA.\textsuperscript{[22]} The effects of chloroform crude extracts of S. nigrum were showed inability to stimulate lymphocytes to divide cells in the six concentrations compared with control.

Colchicines is a drug derived from Colchicum autumnale worked to prevent the formation of spindlefibers yarns and keep dividing cells in the tropical Metaphase, which facilitates the accumulation phase and could studded.\textsuperscript{[23]} When using extracts as an alternative material suspended divisive shown there was no signal to stopping division in Metaphase as Colchicine worked.

**Table (1): Phytochemicals detection chloroform crude extracts of S. nigru.**

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Black Berries</th>
<th>Green Berries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Comarins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

The extract contains the designated phytochemicals.+)

(-) The extract dose not contains the designated phytochemicals.

**Figure (1): The effect of chloroform extracts of S. nigrum black berries on SR cell line growth inhibition (GI %) in different concentrations and at three times exposure.**
Figure (2): The effect of chloroform extracts of *S. nigrum* green berries on SR cell line growth inhibition (GI %) in different concentrations and at three times exposure.

Table (2): The dose-effect parameters (IC$_{50}$) for chloroform berries extracts of *S. nigrum* on SR cell line.

<table>
<thead>
<tr>
<th>Types of Berries</th>
<th>$m$</th>
<th>Dm$_{µg/mL}$</th>
<th>$r$</th>
<th>$M$</th>
<th>Dm$_{µg/mL}$</th>
<th>$R$</th>
<th>$m$</th>
<th>Dm$_{µg/mL}$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>0.437±0.040</td>
<td>4133.7</td>
<td>0.983</td>
<td>0.496±0.011</td>
<td>1358.3</td>
<td>0.999</td>
<td>0.714±0.037</td>
<td>669.3</td>
<td>0.995</td>
</tr>
<tr>
<td>Green</td>
<td>0.444±0.038</td>
<td>6571.7</td>
<td>0.986</td>
<td>0.435±0.014</td>
<td>1787.2</td>
<td>0.998</td>
<td>0.691±0.063</td>
<td>1244.0</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Dose-effect parameters:

$m$: Is the shape coefficient of the sigmoidicity of the dose-effect. $m=1$, $m>1$ and $m<1$ indicate hyperbolic, sigmoidal and negative sigmoidal dose-effect, respectively.

$Dm$: Is the dose for 50% effect (e.g., 50% inhibition of bioluminescence).

$r$: Is the value of liner regression correlation coefficients.

Figure (3): The effect of chloroform extracts of *S. nigrum* black and green berries on REF cell line growth inhibition (GI %) in different concentrations and after 72h of exposure.
Table (2): The dose effect parameters (IC\textsubscript{50} value) of chloroform extracts on REF cell line.

<table>
<thead>
<tr>
<th>Types of Berries</th>
<th>dose effect parameters (IC\textsubscript{50} value)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>Dm µg/mL</td>
<td>r</td>
</tr>
<tr>
<td>Black</td>
<td>0.486 ± 0.049</td>
<td>9530</td>
<td>0.981</td>
</tr>
<tr>
<td>Green</td>
<td>0.454 ± 0.025</td>
<td>7628.1</td>
<td>0.994</td>
</tr>
</tbody>
</table>

DISCUSSION

This study showed that chloroform fruits extracts of \textit{S. nigrum} have cytotoxic activity on cancer cell line (SR) Non Hodgkin Lymphoma. The mechanism of growth inhibition for cancer cells occurs through many pathways such as apoptosis. The mechanisms of Saponins were Induced apoptosis and autophagy act as antimicrotubule agent induction of endoreduplication and mitotic arrest suppressing MMP-2 and MMP-9 production activation of caspase 2.\cite{24} Extracts of \textit{S. nigrum} have significant anticancer activity against numerous cell types. Some of alkaloids were approved as antineoplastic agents to treat Leukemia, Hodgkin's disease, malignant lymphomas, Neuroblastoma, Rhabdomyosarcoma, Wilms tumour. Alkaloids arrest cancer cell proliferation by binding to tubulin in the mitotic spindle, they also induce apoptosis.\cite{25}

The extracts did not affect the preparation of lymphocytes, perhaps the reason for this was that the materials in these extracts do not stimulate the cells to divide. They also do not have the ability to stimulate the production of immune proteins of lymphocytes, so could not works to stop the division of lymphocytes at the equatorial phase. This study proved that chloroform green. The overall study evaluate that \textit{Solanum nigrum} has potential activity on SR cell line, but less effect on REF cell so these extracts has considerable anticancer activity Non Hodgkin Lymphoma.

REFERENCES


