In Vitro Cytotoxicity of Total Alkaloid Extract from Peganum Harmala L. Seeds

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Abstract

Objectives: (Investigation the cytotoxicity of total alkaloid extract of Peganum harmala L. seeds against tumor cell lines. Methods: From the seeds of P. harmala, total alkaloid was extracted using 80% methanol, chloroform (at pH2 and pH10 and they chloroform part was dried to get the extract of totaly alkaloid. The totaly alkaloidss were revealing of qualitatively by Dragendorff’s, Mayer’s and Hage. r’s) reagents and estimated quantitatively by Bromo cresol green spectro.photometry depending on the curve of atropine calibration. (The activity of cytotoxicity was achieved by using Michigan Cancer Foundation-7 (MCF-7) breast cancer cell line and fetal hepatic cell line (WRL-68) non-tumorigenic by MTT assay. Resultse: The total content of alkaloid of P. harmala extract was 328.62± 2.8 mg/100 g dry weight of plant. This extract drop the viability of cells in both cell lines, the greatest reduction happened in the concentration 400 μg/ml was 60.2± 2.8 % for MCF-7 and 66.5±2.2% for WRL-68. Conclusion: (The alkaloids of P. harmala had variable effects against cancer and normal cell lines depending on the type of alkaloid compounds and their concentration in the extract).

Keywords: Alkaloids, P. harmala, In vitro, Cytotoxicity, Breast cancer.

Introduction

One of the most life-threatening diseases is cancer and have many health in developing represented by irregular proliferation of cells. (The toxicity of chemotherapeutical medication typically creates a big drawback within the treatment of cancer exploitation medical or established drugs, Plants still have monumental potential offer to produce newer medication and intrinsically area unit a reservoir of natural chemicals which will provide chemo.protective potential against cancer. (Recently varied therapies are propounded for the treatment of cancer, several of that use plant-derived product. The medicine is always playedj an important role within the treatmenf of disease. Plants have considerable potential for plant-based therapies. The demand for plant-based medicines, food supplements, health products, prescribed drugs and cosmetics square measure increasing in each developed and developing countries because of the versatility medicinally and environmentally.

Secondary metabolites are developed in nature’s variety of completely different plant species, insects, fungi, algae an and other existing organisms, as a consequence, some represent important therapeutic agents. In fact, alkaloids are among the most vital active parts in plants, and a few of those compounds have already been with success developed into therapy medication. Like camptothecin (CPT), a famed topoisomerase I (Top I) inhibitor, and l-2vinblastine, that interacts with tubulin. (Many alkaloids exhibit important biological activities, like the relieving action of ephedrine for bronchial asthma, the analgesic action of morphine, and the anticancer effects of vinblastine). The target of our study was to analyze the cytotoxic
activity of total alkaloid extract of Peganum harmala L. against 1 breast cancer cell line Michigan Cancers Foundation (MCF-7) and non-tumorigenic fetal 1 hepatic cell line (WRL-680).

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Plants Collection

The Peganum harmala seeds were collected from the cultural space in Babylon Province, Iraq, throughout March 2019. The plant seeds were washed with tap water to get rid of dirt and so with distilled water (DW), and dried below shade for several days at room temperature. The seeds were ground and keep in air-light container to forestall the humidness impact and so hold on at room temperature till additional use.

Extraction of total alkal0id

Total alkaloids were extracted in keeping with Harborne. Briefly, 120g of dry plant powder was extracted with 80% methanol for 124 h in continuous extraction by Soxhlet apparatus 250 ml volume. (The extract was filtered by Whatmani No.11 filter paper and then, the filtrate was concentrated by a rotary evaporator below vacuum at 45°C until the solution reached 10 ml). Subsequently, the concentrated extract was transferred to a separating funnel and 1 HCl was added step by step to regulate the pH value up to two, at that time the extract was washed with ten ml chloroform three times. Then, the pH value of the extract was adjusted to neutral with 0.1 NaOH. Then, 15 ml of BCG solution and 15 ml of phosphate buffer were added to the extract. The mixture was shaken and the complex was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking, the extract was then collected in a 110 ml volumetric flask and diluted with chloroform. They absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

Detection of qualitative alk0l0ids

Some qualitative tests were performed too detect the presence of alkaloids in plant extract by using Mayer’s, Dragendorff’s, and Hager’s reagents. (1Mayer’s reagent used to screen all types of alkaloids, 1prepared by dissolving 113.5 gi of Mercurice chloride and 15 gi of Klin 1000 ml DIW). Thei tests was done by adding 11-2 ml of thei reagent to 15 ml of plant extract. Thei formati0n 0f a prominent white precipitate indicated thei test was positive. 1Hager’s test, 1Hager’s reagent i saturated solution of Picric acid, was done by adding a few drops of the reagent to plant extract, the formation of a yellow color indicated the test was positive. 1Bayer’s solution (1mg/10 ml) and each of them was transferred to separate funnels and washed with 10 ml chloroform (3times). (They pH 0 of thei extract was adjusted to neutral with 0.1 NaOH. Theni 15 ml 0f BCG solut0n andi 15 ml 0f phosphate bufferi werer addedi t0 they extracte). Thei mixturei was shaken and the complex was extracted with thei, 2, 3, and 4 ml chloroform by vigorous shaking, they extracte was theni collecteci in a 110 ml volumetric flask and diluted with chloroform. They absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

Estimations of total alk0l0id content

They total alkaloid content was calculated by Bromocresol Greeni (BCG) spectrophotometry technique. (They BCG reagent was prepared by heating 169.8 gi of bromocresol green with 13 ml of 12N NaOH and 5 ml D,W till fully dissolved and so, they solution was diluted to 1000 ml with DIW). 1Phosphate bufferi solution (1pH 4.17) was prepared by adjustingi the pH 0f 2Mi 1sodium phosphate (171.6 gi Na₂HPO₄ in l DIW) t0 14.7 withe 0.12 Mi citricy acide (142.0 gi citrice acide in l DIW). 1BCG lassay: A 1o mg of the plant extracte was dissolved in 2N iHCl andi thenn filteredi. Thise solution (1ml) was transferred to a separating funnel and washed with 10 ml chloroform (3times). (They pH 0 of thei extract was adjusted to neutral with 0.11 NaOH. Theni 15 ml 0f BCG solut0n andi 15 ml 0f phosphate bufferi werer addedi t0 they extracte). Thei mixturei was shaken and the complex was extracted with thei, 1, 2, 3, and 4 ml chloroform shakinng, they extracte was theni collecteci in a 110 ml volumetric flask and diluted with chloroform. They absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.
Cytotoxic activity

To determine the cytotoxic activity against two kinds of cell lines including breast cancer cell line 1MCF-17 and non-mutagenic fetal hepatocyte 1WRL-68 using 13-[14, 15 – dimethylthiazoyly]-12, 15-diphenyltetrazolium bromide (MTT1 dye). (Briefly), 1100 µl cell suspension was added into culture plate wells, separated plates for each cell line in triplicate, and treated with 100 µl partially purified plant extract, incubated for 12 hr, centrifuged to remove dead cells. Aliquot of 100 µl of 12 mg/ml MTT dye was added to each well and the incubation was continued for a further 4 hr, then 50 µl of 1% solubilization solution of DMSO was added into each well. After complete solubilization of the dye, the absorbance of MTT assay was read at 492 nm with an ELISA reader. The percentage viability of cells exposed to various treatments was calculated as followst:

The control was the non-treated cultures in all experiments that contained cells in the medium only. (This assay was held at the Centre for Natural Product Research and Drug Discovery, Department of Pharmacology, Faculty of Medicine, University of Malaya / Kuala Lumpur, Malaysia).

Statistical Analysis

(Statistical analysis of the data was performed by using SPSSII 14.0 version using one way analysis of variance (ANOVA) according to the methodology described by Levesque 16 (1numerical) data were expressed as mean ± SD. P < 0.05 were considered to be statistically significant.

Results and Discussion

The qualitative analysis of Peganum harmala seed extract appears the presence of alkaloids by changing the color in each reagent (Table 1). The quantitative content of alkaloid compounds in the P. harmala seeds extract was 328.62 ± 2.8 mg/100 g DW. The results of cell viability assay based on the MTT assay using 1MCF-7 and WRL-68 cell lines which treated with total alkaloid extract of Peganum harmala seeds appeared the percentage of cytotoxicity increased with increasing concentration of alkaloids (Table 2). Also, it had a cytotoxicity effect on both cancer and normal cell. The highest reduction of viability was observed at the highest concentration (400 µg/ml) of Peganum harmala alkaloid extract was 60.2 ± 2.8% for MCF-7 and 66.5 ± 2.2% for WRL-68.

Table 11: Qualitative detection of P. harmala alkaloid extract using different reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Result</th>
<th>Result e00r</th>
</tr>
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<tbody>
<tr>
<td>Mayer’s reagent</td>
<td>+</td>
<td>Creamy precipitate</td>
</tr>
<tr>
<td>Dragendorf’s reagent</td>
<td>+</td>
<td>Orange color</td>
</tr>
<tr>
<td>Hager’s test</td>
<td>+</td>
<td>Yellow color</td>
</tr>
</tbody>
</table>

Figure 1: Calibration curve of the atropine using BCG methods at 470 nm

Table 2: Cytotoxic activity of the total alkaloids of P. harmala L. against the breast cancer cell line MCF7 and normal cell line 1WRL-68.

<table>
<thead>
<tr>
<th>Alkaloid extract conc. µg/ml</th>
<th>%Viability of WRL± SD</th>
<th>%Viability of MC7± ISD</th>
<th>1IC50 of MC7 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1400</td>
<td>66.5 ± 12.2</td>
<td>60.2 ± 12.8</td>
<td>5.19e+006</td>
</tr>
<tr>
<td>1200</td>
<td>85.16 ± 1.50</td>
<td>78.90 ± 1.30</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>88.50 ± 2.00</td>
<td>85.14 ± 4.00</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>93.12 ± 0.70</td>
<td>96.05 ± 2.90</td>
<td></td>
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</tbody>
</table>

(The alkaloids are the most active principles present in the seeds of P. harmala. Extraction of total alkaloids from the seeds of Peganum harmala plant has achieved a high yield, but still a low yield) compared to bibliographic data reported by Bukhari et al. 17. That it could be explained by the use of a different...
The range of alkaloid concentration necessary to elicit the anticancer effects is widely and not all alkaloids can react with 1BCG dye. Therefore, due to their lack of a general method to estimate all types of alkaloids, the method described in this study can be used for their determination of a special group of alkaloids. 1BCG can react with a certain class of alkaloids and some alkaloids do not react with this reagent.

P. harmala has been used in traditional medicine, but remains a poisonous plant for humans and animals. So, the alkaloid extract of P. harmala seeds had anticancer activity to reduce the growth of cancer cell, also it had inhibited effect on normal cell. This plant is a rich source of β-carboline alkaloids, which constitute the majority of alkaloids of Peganum harmala. (The compounds that inhibit cancer initiation are typically termed (blocking agents), thus biologically active compounds present in plants can prevent carcinogenesis by blocking metabolic activation, increasing detoxification), or providing alternative targets for electrophilic metabolism. (They may act by preventing the interaction between chemical carcinogens or endogenous free radicals and DNA, thereby reducing the level of damage and resulting in mutagens which contribute not only to cancer initiation but also to progressive genomic instability and overall neoplastic transformation). Protection may be achieved as a consequence of decreased cellular uptake and metabolic activation of pro-carcinogens but also by enhanced detoxification of reactive electrophiles and free radicals scavenging, as well as induction of repair pathways. (This activity of inhibition may be due to the nature of the compounds that inhibit cellular uptake and their interaction with metabolic nature of each type of cancer cell). Some may be due to the effectiveness of some enzymes that act as antioxidants especially in cancer cells.

**Conclusion**

Plant alkaloids had variable effects against cancer cell and normal cell lines depending on the type of alkaloid compounds and their concentration in the extract. Also, these alkaloids need further purification and tested against different cell lines to determine their effectiveness.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Biology and all experiments were carried out in accordance with approved guidelines.

**References**


