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, chloroforme(at pH2 and pH10 and they chloro form partn was dried to gettjing the extract ofy totaly alkaloidd t. The totaly alkaloidss werey revealing ofd 1qualitatively by Dragend0rff’s, Mayer’s and Hage.r’s) 1reagents and estimated 1quantitatively by Bromo cresol green spectro.photometry depending on the curve of atropine 1calibration. (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 assayi. Resultse: The total content of alkaloid of *P. harmala* extract was 328.62± 2.8mg/100 g dry weight of plant. This extract drop the viability of cells in b0th 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 againstt cancery andy normale cellt linesy 1depending 0n thei type of alkal0id compounds andi 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 care 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 1cancer ¹. (Recently varied therapies are propounded for the treatment of cancer, several of that use plant-derived product. The medicinest alwaysy playedj an imp0rtant r0le within the world healthg). Thed healthd 1medicinal 1plants pr0viding av replacement space ofs 1drug analysis ². ¹The demanda for planty primarily basedt 1medicines, f00d supplementy, health pr0duct, prescribed drugs andy c0smetics square measure increasingi ini each 1developing 1and develop0ped c0untries because 0f ther 1growing recogniti0n that then a naturale product aret non-t0xic, have less side effects and simply out there ³. (Secondary metabolites are developed in nature’s variety of completely 1different plantd speciess, insectsi, fungal, algae an Andy pr0kary0tes throughout their courses 0f evoluti0n in monumental diversity). Plantr sec0ndary metab0litess cani bei defined asthe 1compounds thatt playedi a vital r0le within the interactioin of thei plante within it surr0unding, however haven’t any such role in maintaining the basic life processes in plants ⁴. (The alkaloids represent a bunch of natural product that has had a significant impact throughout history on the economic, medical, political and social affairs of humans). Several of those agents have potent physiol0gical effects on mammalian systems moreover as different organisms, and as a consequence, some represent vital therapeutic agents ⁵. In fact, alkal0ids are among the most vital active parts in plants, and a few of those 1compounds have already been with success developed int0 therapy medication. like camp0thecin (CPT), a famed topois0merase I (Top I) inhibit0r ⁶, and 1vinblastine, that interacts with 1tubulin ⁷. (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) ⁸. The target of our study was to analyze the cytotoxicity...
activity of total alkaloid extract of *Peganum harmala* L. against breast cancer cell line Michigan Cancers Foundation-71 (MCF-7) and non-tumorigenic fetal liver hepatic cell line (WRL-680).

**Materail and Meth0d**

**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 alkaloid**

Total alkaloids were extracted in keeping with Harborne. Briefly, 120g of planta dry powder was extracted with 80% methanol for 124 hina continuous extraction by S0xhlet apparatus 250 ml volume. (The extract was filtered by Whatmani No.11 filter paper andy theni, therr filtrate wass c0ncentrated bya r0tary evap0rator bel0w vacuumy at45Cº untill they s0lution reached in 10 ml). Subsequently, thei c0ncentrated extractt was transferred t0 ia 1separating funnel and 12 Ni 1HCl wast added step by step t0 regulate thei pH valuee iup t0 two, at that time thei extract was washed 1with ten mll chl0r0f0rm thrice. Theni ,thei 1pH valuo 0f thei extracts was adjusteed t0 ten using 1NH4OH, andi partiti0ned 1with ten mll chl0r0f0rm trice. The chlor0form p0rtion wast driedt to get they overall alkaloid extract. They dried extract wast weighd, 1and preserved ini a cleann c0ntainer att four °C1 f0r further investigati0n.

**Detection of qualitative alkaloids**

Some qualitative tests were performed too detect the presence of alkaloids in plant extractsi by using (1Mayer’s, Dragend0rff’s 1and 1Hager’s reagents). (1Mayer’s reagenti used t0 screen alltypes 0f alkaloids, 1prepared biy disolv1ng 113.5 gi 0f Mercurice chl0r0ide andi 15 gi 0f Klin 1000 mll DIW). Thei tests wast d0ne bye addiing 11-2 mll 0f thei reagentt t0 15 mll 0f plante extracte. They f0rmati0n of a whitee precipitat indicatedi thei test wast p0sitive 10. Also, 1Dragendorff’s reagenti wast usedi t0 investigatei alkaloidis inplante extracte. (They reagenti wast preparedj bye disollving 2o gi 0f Bismuthe Nitrate ini 140 mll DIW andi 116 gi 0f S0dium I0dide int 40 mll DIW, thene they tw0 soluti0ns were mixedd t0gether). They teste wast perf0rmed bye ading 11-2 mll 0f Drangen0rff’s 1reagenti int 15 mll 0f the plante extractt, 1the f0rmati0n of a pr0minent 0range c0llor indicatedi thei 1test wast wash p0sitive. 1Hager’se teste, 1Hager’s reagenti isi saturatedi s0lution 0f Picrice acide, wasd d0ne bye ladding afew dr0ps of the reagenti t0 plante extracte andi appearedd ayellow c0llor precipitatei thati indicatei tothe presenceei 0f alkaloidis).

**Estimations of total alkalioid content**

Thei t0tal alkaloid contente wast calculable bye Brom0cresol Greeni (BCGi) spectro photometry technique (13 , 14). (They iBCG reagentt wast preparedi bye heatingi 169.8 mg 0f brom0cresol greenl withi 13 mll 0f 12N NaoH andi 5 m1 D,W til filly diss0lved andi s0, they s0lution wast diluted t0 1000 mll withe DIW). 1Phosphate bufferi s0lution (1pH 4.17) wast preparedi byy adjustiing the pH to 4 withi 0.12 Mi citracy acide (142.02 gi citrice acide in1 l DIW).

1BCG lassay: A 1o mgi ofthe plante extracte wast dissolv3d in2N hiCl andi thenn filterdi. This solution (1ml) wast transferredi t0 separat0r funnell andi washedd withe 10 mil chlor0f0rm (3itimes). (They 1pH 0f thei extracte wast adjustedi t0 neutrale withi 0.11 Ni NaoH. Theni 15 mll 0f 1BCG soluti0n andi 15 mll 0f ph0sphate bufferi werer addeddi t0 they extracte). Thei mixturei wast shakeni andthe c0mplex wast extrected dithe 1ml chlor0fo rm wasdriedt to get they overall alkalioid 1extract. They driedd extractt wast weightd, 1and preservedi ini a cleann c0ntainer att four °C1 f0r furtheri investigati0n.

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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-168 using 13-[14, 15 – dimethylthiazoly]-12, 15-diphenyltetrazolium bromide (MTT1 dye). (Briefly), 1100 µl cell suspension was added onto flat-bottomed micro culture plate wells, separated plate for each cell line in triplicate, and treated them with 100 µl partially purified plant extracts, incubated for 124 h, centrifuged to remove the dead cells. Aliquots of 100 µl of 12 mg/ml MTT dye was added to each well and the incubation was continued for a further 4 h, then 50 µl of 1% solubilization solution of D.MSO was added into each well. After complete solubilization of the dye, the absorbance of each group was calculated. The percentage viability of cells exposed to various treatments was calculated as follows:

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 Products 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 SPSS version using one way analysis of variance (ANOVA) according to the method described by Levesque (2016). 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 appeared 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 1: Qualitative detection of P. harmala alkaloid extract using different reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Result</th>
<th>Resulted color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayer’s reagent</td>
<td>+</td>
<td>Creamy precipitate</td>
</tr>
<tr>
<td>Dragendorff’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</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>
</tr>
</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. (2017). Thats it could be explained by the use of a different
The range of alkaloid concentration necessary to elicit the anticancer effects is wide (6, 7) and not all alkaloids can react with 1BCG dye (14). Therefore, due to their lack of a general method to estimate all types of alkaloids (18), their method described in this study can be used for they determination of a specific group of alkaloids (13, 19, 20). The 1BCG can react with a certain class of alkaloids and some alkaloids do not react with this reagent (14, 21).

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 (17). (The compounds that inhibit cancer initiation are biologically active agents, this biologically active components present in plants can prevent carcinogenesis by blocking metabolic activity, increasing detoxification, or providing alternative targets for electrophilic metabolites.) (They may act by preventing the interaction between chemical carcinogens or endogenous free radicals and anti reactive intermediates that participate in carcinogenesis, thereby reducing the level of DNA damage and resulting in mutagens which contribute not only to cancer initiation but also to progressive genomic instability and overall neoplastic transformation.) Protective may be achieved as a consequence of decreased cellular uptake and metabolic activation of pro-carcinogens but also progressed genotoxic instability and overall non-neoplastic transformation. Protection may be achieved as a consequence of decreased cellular uptake and metabolic activation of pro-carcinogens but also enhanced detoxification of reactive electrophiles and free radicals scavenging, as well as induction of repair pathways (23–25). (This activity of inhibition may be due to the nature of the compounds and their interaction with metabolic nature in each type of cancer cell(s) or may be due to their interaction with metabolic nature in each type of cancer cell(s). It is very important to investigate the activity of these agents against different cell lines to determine their effectiveness.)

**Conclusion**

Plant alkaloids had variable effects against cancer and normal cell lines depending on the type of alkaloids and their concentration in the extract. Also, these alkaloids need to 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 by the Department of Biology and all experiments were carried out in accordance with approved guidelines.

**References**

15. Chih PL, Wei JT, Yuang LL, Yuh CK. The extracts from Nelumbonucifera suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells. Life Sci 2004; 75: 699-16.