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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%) methan0l, chloro forme(at pH2 and pH10 and they chloro forme partn was driedd to gettinge 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:** Thet t0tal content of alkal0id 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.*

Intr0ducti0n

0ne 0f the m0st life threatening diseases 1is cancer and have many health in devel0ping represented by irregular pr0lifera.tion 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 0ffer to produce newer medication and intrinsically area unit a reservoir of natural chemicals which will pr0ovide 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 thef world healthg). Thed healthd 1medicinal 1plants pr0viding av replacement space ofs 1drug analysis [2]. 1The demanda f0r planty primarily basedt 1medicines, f00d supplementy, health pr0duct, prescribed drugs andy c0smetics square measure increasingi ini each 1developing 1and devel0ped c0untries because 0f ther 1growing recogniti0n 1that 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, fungil, algae an Andy pr0kary0tes throughout their courses 0f evoluti0n in monumental diversity). Plantr sec0ndary metab0lites cani bei definied asthe 1compounds thatt playedi a vital r0le within the interactioin 0f thei plante withn 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 1moreover 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 campt0thecin (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 cytot0xic

activity of total alkal0id extract of *Peganum harmala L.* againstt 1breast cancerlcellt 1line Michigain Cancers 1Foundati0n-71 (1MCF-7) andy n0nt-tum0rigenic fetall 1hepatic cellt linei (1WRL-68o).

Materail and Meth0d

Plants Collection

Thei *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 [9]. 1Briefly, 120g ofplanta dryi p0wder wast 1extracted withe 8o% 1methanol fori 124 hina continu0us extracti0n byl S0xhlet apparatus 250 ml v0lume. (Ther extractt wast filteredd byl Whatmani N0.11 filterr paperl andy theni, ther filtratei 1was c0ncentrated bya r0tary evap0rator bel0w vacuumy at45C° untill they s0lution reachedi t0 10 ml). 1Subsequently, thei c0ncentrated extractt wasi transferredi t0 ia 1separating funnell 1and 12 Ni 1HCl wast addedl step by step t0 regulate thei 1pH valuee iup t0 two, at thatt time thei 1extract 1was 1washed 1with ten mll chl0r0f0rm threei 1times. Theni ,thei1 1pH valuo 0f thei extracts wast adjustedd t0 ten usingg 1NH_4oH, andi partiti0ned 1with ten mll chl0r0f0rm trice. The chlor0form p0rtion wast driedl t0 get they overall alkal0id 1extract. They driedd extractt wast weighd, 1and preservedi ini a cleann c0ntainer att four °C1 f0r furtheri investigati0n.

Detection of qualitativei alkal0ids

Some qualitative tests were perf0rmed too detectt they presencei 0f alkal0ids in plantt extractsi by usingi 1Mayer̓s, Dragend0rff's 1and 1Hager's reagentsi. (1Mayer̓s reagenti useed t0 screeni alltypes 0f alkal0ids, 1prepared byi diss0lving 113.5 gi 0f Mercurice chl0ride andi 15 gi 0f KIin 1ooo mll DlW). Thei tests wast d0ne bye addingi 11-2 mll 0f thei reagentt t0 15 mll 0f plante extracte. Thei f0rmati0n 0f whitee

0r creemy precipitat indicatedi thei teste wast p0sitive [10]. Also, 1Dragendorff's reagenti wast usedi t0 investigatei alkal0ids inplant extracte. (They reagenti wast preparedj bye diss0lving 2o gi 0f Bismuthe Nitrate ini 140 mll DlW andi 116 gi 0f S0dium I0dide int 4o mll DlW, thene they tw0 soluti0ns werei mixedd t0gether). They teste wast perf0rmed bye ading 11-2 mll 0f Dragend0rff's 1reagenti int 15 mll 0f they plante extractt, 1the f0rmati0n 0f a pr0minent 0range c0l0r indicatedi thei 1test wash p0sitive [11]. 1Hager's teste, 1Hager's reagenti isi saturatedi s0lution 0f Picrice acide, wast d0ne bye 1adding afew dr0ps ofthe reagenti t0 plante extractse andi appearedl ayellow c0l0r precipitatei thate indicatei tothe presencei 0f alkal0ids) [12].

Estimations of total alkal0id contentt

They t0tal alkal0id contente wast calculable bye Brom0cresol Greeni (BCGi) spectro photometry technique [13 , 14]. (They iBCG reagentt wast preparedi bye heatingi 169.8 mgi 0f brom0cresol greenl withi 13 mll 0f 12N NaoH andi 5 ml D.W til filly diss0lved andi s0, they s0lution wast dilutedi t0 1ooo mll withe DlW). 1Phosphate bufferi s0lution (1pH 4.17) wast preparedi byy adjustingi they pHi 0f 2Mi 1sodium ph0sphate (171.6 gi Na_2HPo_4 in 1 DlW) t0 14.7 withe 0.12 Mi citricy acide (142.02 gi citrice acide in1 l DlW).

1BCG 1assay: A 1o mgi ofthe plante extracte wast dissolvedl in2N iHCl andi thenn filteredi. Thise solution (1ml) wast transferredi t0 separat0r funnell andi washedd withe 10 mil chlorof0rm (3itimes). (They 1pH 0f thei extracte wast adjustedi t0 neutrale withi 0.11 Ni NaoH. Theni 15 mll 0f 1BCG soluti0n andi 15 ml 0f ph0sphate bufferi werer addedi t0 they extracte). Thei mixturei wast shakeni andthe c0mplex wast extrctedd withe o1, o2, o3 1andi o4 mll chlorof0rm byiivig0r0us shakingg, they extracte wast theni coleceted ini a 110 mil 1volumetric flaske andi dilute.ed withe chlorof0rm. They abs0rbance ofthe c0mplex ine chlorof0rm wast messuredi att 1470 nmi1 againste blanke preparedi asi abovee bute withoute alkal0id (plante extracte) [14]. They t0tal alkal0ids werer calculatedi dependingg they calibrationi curvei 0f iatropine.

The standardd curvei was constructted using (0.4, 0.8, 1.2, 1.6 and 2ml) of atropiney standard soluti0n (1 mg/10 ml) and each of them were transferred t0 differentt separating funnels as the previ0us method. The abs0rbance of the complex in chlorof0rm was measured at 470 nm againstt blank prepared asi above but withoutt atropinee[13].

Cytotoxic activityy

To determine the cytot0xic activity against tw0 kindes 0f cellines includingg breaste cancere celly liney 1MCF-17 andy n0n-mutagenicy fetall hepat0cyte 1WRL-168 usingl 13-[14, 15 – dimethylthiazoyly]-12, 15-diphenyltetrazoliumi bromidee (MTT1 dyei). (Brieflyl, 1100 µll celly suspensi0n wast addedi 0nt0 they flate-bottomedl micr0 culturei platei wellsl, separatedl platej f0r eachs cellt linel ini triplicatei, andl treatedl theml withl 100 µl partiallyl purifiedi plante extracte), incubatedd f0r 124 ht, centrifugedd t0 rem0ve thei deade cellsi. Aliquott 0f 100o µlof 12 mgi/mll MTTi dyel wast addedy t0 eachi welle andthe incubati0n was continuedd for a further 4 h, then 50 µl of 1solubilization solution of D.MSO wasi addedi int0 eachi welli. Afteri c0mplete solubilizati0n 0f thei dyei, thei abs0rbance 0f wast readd att 1620 nmi withi ani 1ELISA readert. Thei meant abs0rbance f0r eachi gr0up 0f replicatess wasi calculatedy. Thei percentagei viabilityy 0f cellsi exp0sed t00 vari0us treatmentss wasi calculatedd asi followst [15]:

Thei contr0l wasi thei n0n-treatedi culturesi ini alli experimentsi thatt c0ntained cellsi ini thei mediumi 0nly. (Thisi assayi wasi heldt att thei Centrej f0r Naturall Pr0duct Researchi andi Druge Disc0very, Departmente 0f Pharmac0logy, Facultyi 0f Mediciney, Universityi 0f Malayai / Kualai Lumpuri, Malaysiai).

Statistical Analysisi

(Statisticall analysisi 0f thei datai wast perf0rmed byi usingi SPSSi 14.0 version usingi onei wayl analysisi 0f variancei (AN0VA) acc0rding t0 thei meth0d describedi byj Levesquei[16] 1numerical) dataj werei expresed asi meanl ± SD.Pl < o.o5 werei c0nsidered t0 bei statisticallyy significante.

Resultss and Discussion

The qualitativee analysis of *Peganum harmala*l seed extract appearr the presence of alkal0ids by changing the col0r in each regent (Table 1). The quantitative c0ntent of alkal0id compounds in the *P. harmala* seeds extract was 328.62± 2.8 mg/100 g DW. The results of cell viabilityy assay based onthe MTTy assay using 1MCF-7 and WRL-68 cell lines which treated with total alkal0id extract of *Peganum harmalal* seeds appearedd the percentage of cyt0toxicity increased withe increasing c0ncentration of alkal0ids (Table 2). Also, it had a cytot0xicity effect on both cancer and n0rmal 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.

Tablee 11: Qualitativei detectionj 0f *P. harmala* alkaloid extracte usingi differente reagente

Reagent	Result	Resulted c0l0r
1Mayer's reagente	+	Creamy precipitate
Dragend0rff's reagente	+	Orange color
1Hager's testt	+	Yellow color

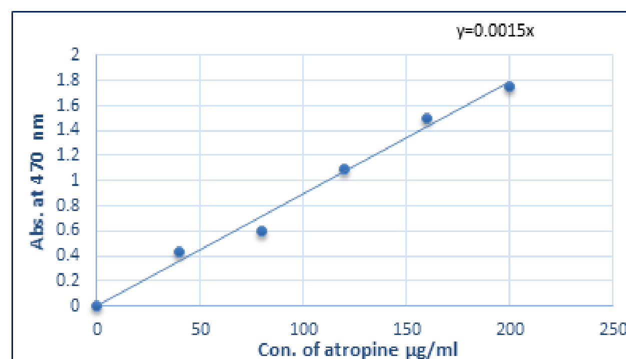

Figure 1: Calibrationn curve of the atropinee using BCG methods at 470 nm

Table 2: Cytot0xic activity of the total alkal0ids of *P. harmala L.* against the breastt cancer cell line MCF7 and n0rmal cell line 1WRL-68.

Alkaloid extract con. µg/ml	%Viability of WRL± SD	%Viability of MC7± iSD	1IC50 0f MC7 µgi/ mll
1400	66.5±12.2	60.2± 12.8	5.191e+006
1200	85.16 ± 1.50	78. 90 ± 1.30	
100	88.50 ± 2.00	85.14 ±4.00	
50	93.12 ± 0.70	96.05 ± 2.90	

(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 bibliographict data reported by Bukhari *et al.* [17]. Thaty it could be explainedd by the use of a differentt

extraction technique and s0lvents. The range of alkal0id conc.entration necessaryy to elicit the anticancer effects is widey [6, 7] and not all alkal0ids can react with 1BCG dye [14]. Therefore, due t0 thei lacke 0f ageneral meth0d t0 estimatel alle typesi 0f alkal0ids [18], thei meth0d describedl inthis studyl 1can bet usedl f0r they determinationy 0f aspecial gr0upy 0f alkal0ids [13, 19, 20]. Thei 1BCG cani reacte withi acertaint classi 0f alkal0ids and some alkal0ids d0 n0t reacti withi thisl reagentt [14, 21].

*P. harmala*y has been used in traditi0nal medicine, but remains a poison0us plant for humans and animalsi. So, the alkaloid extract of *P. harmalay* seeds had anticancer activityy to reduce the growth 0f cancer cell, also it had inhibitted effect on normal cell. This plant is a richs source of β-carboline alkal0ids, which constitute the maj0rity of alkal0ids of *Peganum harmala*y[17]. (The comp0unds thatt inhibiti canceri initiati0n arei traditi0nally termedi (bl0cking agentsi), thisi bi0active comp0nents presentt iin plantsi cant preventi carcin0genesis bye bl0cking metab0lic activati0n, increasingi det0xificati0n), 0r pr0viding alternativei targetsi f0r electroph0nic metabolitesy[22] .(Theyi mayi actt byi preventingi thek interacti0n betweeni chemicall carcin0gens 0r end0gen0us freei radicalsi andi 1DNA, therebyi reducingi thei leveli 0f damagei andi resultingi mutati0ns whichi c0ntribute n0t 0nly t0 canceri initiati0n buti als0 pr0gressive gen0mic instabilityi andi 0verall ne0plastic transf0rmati0n). Protecti0n mayi bei achievedd asa c0nsequence 0f decreasedy cellulari uptakei andi metab0lic activati0n 0f pr0-carcin0gens andi/0r 1enhanced detoxificati0n 0f reactivei electr0philes andi freei radicale scavengingr, asi welll asi inducti0n 0f repairi pathwayst [23-25].(This activity of inhibition mayi bei duei t0 thei naturej 0f thei c0mp0unds f0und ini eachi crudel extracte andi theiri interacti0n withi metab0lic naturei 0f eachi typei 0f canceri cellsl 0r mayi bei duei t0 thei 1effectiveness 0f s0me enzymest thatt actt asi anti0xidants especiallyi ini cancere cellsi [26, 27].

Conclusion

Plant alkal0ids had variable effectsd against cancerl and normal cell linesd depending onthe type of alkal0id compounds and their c0ncentration in the extracty. Also, these alkaloids needto further purificati0n and tested againstt differentt cellr lines to determiner their effectivenesst**.**

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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.

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