Effect Of Arbutin Peel From Pyrus Communis On Cell Line

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Objective: Arbutin (P-Hydroxyphenyl-B-D-Glucopyranoside) Possesses Beneficial Extracted From Plant *Pyrus Communis* Functions Including Anti-Tumor Activities. Due To The Important Role Of Anti Tumor And Apoptosis In The Successful Treatment Of Cancer, Is Essential. The Purpose Of The Current Study Was To Evaluate The Effect Of Arbutin On Cell Line Hepg2 ,MCSF7 And MEF Cellline .

Materials And Methods In This Experimental Study, Effect Arbutin And Extracted From *Pyrus Communis* In Hepg2 ,MCSF7 And MEF Cell Lines Were Pre-Treated With Arbutin (6.12 ,12.5, 25,50, And 100 Mm). After 24 Hours, T-BHP (30 And 35 Mm) Was Added To The Cells. Viability Was Measured (At 24) Using MTT Assay (3-[4,5-Dimethylthiazol-2-Yl]-2,5 Diphenyl Tetrazolium Bromide).

Results: Arbutin Treatment To Cell Line Assay Including Effect Of Extract On Growth Of Tumor Cell Lines Hepg2, MCF7 And MEF Normal Cell Line Was Done By Using Cytotoxic Assay. The Findings Demonstrated That The Cytotoxicity Of The Extract Varied Depending On Cell Type And Concentration In Different Cell Lines. With Higher Extract Concentrations, Tumor Cell Line Inhibition Activity Increased. The Higher Inhibition Rate In Hepg2 Cell Line Was 92.01 % And 79.87 At The Concentration 100 µg\Ml While For MCF7 The Highest Inhibition Rate Was 62.12% And 59.87in Concentration 100 µg\Ml The Highest Inhibition Rate For MEF Cell Line Was 59.17 % And 42.81 In 100 µG\Ml

Conclusion: Arbutin As A Potential Functional And Extracted Has Strong Ability To Inhibitory Effect On The Growth Of Cell Line And Decreased Effect In Normal Cell Line .

Keywords: Arbutin; MEF Cell Line ; *Pyrus Communis*. Hepg2 Cell Line

Introduction

Arbutin (C12H16O7), Also Known As B-Arbutin, Is A Hydroquinone Glucoside (Figure 1). This Compound Was First Reported From The Leaves Of Arbutus Unedo L. (Family: Ericaceae) [1]. Arbutin Structurally Differs From Its Isomer A-Arbutin By The Presence Of A B-Glucose Unit Instead Of

An A-Glucose One. As This Glycoside Is Capable Of Inhibiting Melanin Production By Inhibiting Tyrosinase, It Has Long Been Used As A Skin Whitening (Depigmenting) Agent In Various Commercially Available Topical Cosmetic Products [2]. It Should Be Mentioned Here That Tyrosinase Is A Multi-Copper Enzyme That Plays A Pivotal Role In Melanogenesis And Enzymatic Browning. The Objectives Of This Review Are To Extensively Explore, For The First Time, The Distribution Of Arbutin.

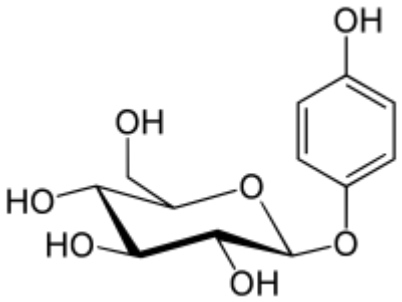

Figure 1. Arbutin

In Addition To Its Skin Whitening Property Which Has Been Known For At Least Seven Decades, Arbutin Has Been Shown To Possess Various Other Therapeutically Relevant Biological Properties, E.G., Antioxidant, Antimicrobial And Anti-Inflammatory [3]; It Also Has The Potential As An Anticancer Agent [4]. Information Obtained From The Published Literature On Arbutin Shows That This Compound Possesses Cytotoxic Properties Against Several Human Cancer And Tumor Cell Lines Including Bladder, Bone, Brain, Breast, Cervical, Colon, Gastric, Liver, Prostate And Skin Cancers,[5]. Most Of These Activities Have Been Demonstrated In Vitro, And In Some Cases, Plausible Mechanisms Of Action, E.G., Apoptosis, Have Been Identified. A Pictorial Summary Is Presented In Figure2. The Activity Of Arbutin Against Various Cancer Cell Lines Is Discussed In The Following

Subsections.[6]

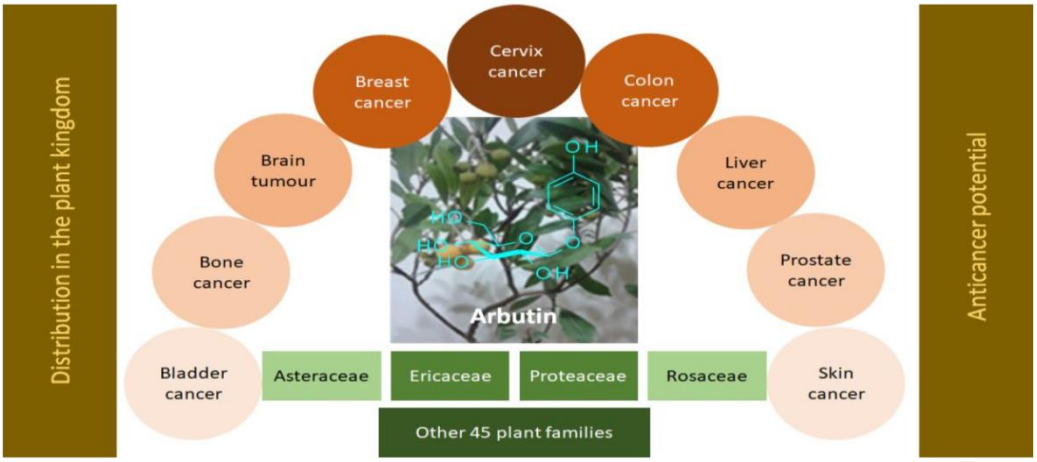

Figure 2. A Schematic Summary Of The Anticancer Potential Of Arbutin, Obtained From Different Plant Families.

Apoptosis Is A Complex Programmed Cell Death, Manifesting As Cell Shrinkage, Chromatin Condensation And Internucleosomal DNA Fragmentation [7]. Extrinsic And Intrinsic Pathways Play Important Roles In Cellular Functions, And Mitochondrial Function Is Considered To Be A Therapeutic Target For Cancer Treatment [8].

Breast Cancer Is The Most Common Type Of Cancer And Is Usually Treated With Chemotherapy And Radiotherapy [9]. In The Search For Natural Products As Potential Cures For Breast Cancer, The Cytotoxicity Of An Arbutin-Containing Methanol Extract Of Turnera Diffusa Was Evaluated Using The MTT Assay Against Epithelial-Like MDA-MB-231 Breast Cancer Cells; The IC50 Value Was Determined To Be 30.67 Mg/Ml [10].

Liver Cancer Is One Of The Leading Causes Of Cancer Deaths Worldwide And Is The Sixth Most Common Form Of Cancer In Humans, With Almost A Million New Cases In 2020 [5]. Reported In Vivo Anticancer Activity Of Arbutin Against Diethylnitrosamine-Initiated Liver Carcinogenesis In Rats. This Effect Was Attributed To The Anti-Inflammatory And Antioxidant Properties Of Arbutin.

In The Present Study, Arbutin Was Prepared In Order To Improve The Biological Effects Of Arbutin, And The Effects Of Arbutin On MCF-7 Human Breast Cancer Cell Line, Hepg2 Hepatocellular Cancer Cell Line.

Materials And Methods

Preparation Of *Pyrus Communis* Plant Aqueous Extract

Soak 50 G Plant Material In A Liter Of Distilled Water For Two Hours On A Vertical Shaker, Then Left For A While 24 Hours In A Dark Place, Then Filter The Liquid In Two Stages, The First From Through A Muslin Sieve To Get Rid Of The Largest Parts, And Through The Second Blotting Paper, The Resulting Concentration Is 100%, Which Is The Original (Raw) Concentration From Which The Final Concentrations Used Were Made.

Cell Lines

In This Study, The Human Breast Cancer MCF7 Cell Line Was Used In Passage NO. (56), It Was Isolated In 1970 From A 69-Year-Old Woman, Hepatocellular Carcinoma Hepg2 Cell Line Was Used In Passage NO. (57), It Was Isolated In 1975 From A 15-Year-Old, White, Male Youth With Liver Cancer While The Mouse Embryonic Fibroblasts MEF Normal Cell Line Was Used In Passage No. (47).

Measuring Cell Viability Using MTT Assay

Tetrazolium Dye 3- [4, 5-Dimethylthiazol-2-Yl]-2, 5-Diphenyltetrazolium Bromide (MTT) Is Usually Used To Assess Cells Viability. The MTT-Colorimetric Assay Is Based On The Capacity Of Viable Cells To Reduce MTT Into Formazan Dye Through Succinate Dehydrogenase In Mitochondria. After Exposure Of The Cells To Arbutin With/ Without Consequent Exposure To T-BHP And Incubating For 24 And 48 Hours, 50 Ml Of 5 Mg/Ml MTT In PBS Was Added To Each Well And Incubated For Another 4 Hours. Afterward, The Media Were Aspirated, And The Formazan Precipitate Was Dissolved In 150 Ml Dimethyl Sulfoxide (DMSO) To Lyse The Cells. The Color Intensity Of The Solution Was Measured By Camspec-M501 Spectrophotometer (Camspec, UK) At 570 Nm With 630 Nm As The Reference Wavelength. The Results Were Reported As The Percentage Of The Control Ones [11].

Statistical Analysis

One-Way ANOVA With Post-Hoc Test (Tukey) Was Used For Statistical Comparison, And $P<0.05$ Were Contemplated Statistically Significant.

Results

The Effects Of Extract Aqueous Peel From *Pyrus Communis* And Arbutin Peel From *Pyrus Communis* On The MCF7 , Hepg2 And MEF

To Test The Effect Of The Aqueous Peel From *Pyrus Communis* And Arbutin From *Pyrus Communis* Ability In The Growth Of Cancerous

Tumors, The Test Was Conducted On Two Cancer Lines (MCF7 , Hepg2) And Normal Cell Line (MEF). Cancer Cell Lines Were Treated With Five Concentrations And Three Replicates For Each Concentration For 24 Hours At A Temperature Of 37 By Using Different Concentrations That 6.25, 12,5, 25, 50, 75 , And 100 Mg/Ml, And The MTT Test Was Adopted To Determine The Effect Of The Concentrations Of The Extract On The Growth Of The Cells, In A Term Of The Percentage Of The Rate Of Inhibition Of Growth.

Table (1) Showed That The Extract Aqueous Peel From *Pyrus Communis* Had An Inhibitory Effect On The Growth Of Cancer Cells Of The MCF7 Cell Line, Starting With A Concentration Of 6.25 Mg/Ml, As The Percentage Of Inhibition Was 12.98%, And This Percentage Increased To 16.24%, 49.75% ,50.13 % , 56.08% And 59.87%, For The Concentrations Used, Respectively. A Significant Difference Was Observed Between Concentrations.

Table (1) Inhibition Percentages In The MCF 7 Cell Line By The Effect Of Different Concentrations Of The Extract Aqueous Peel From *Pyrus Communis* For A Period Of 24 Hours Exposure At A Temperature Of 37 °C.

Standard Deviation. Inhibition Ratio100%	Con . Mg/Ml
1.0 ± 12.98 B	6.25
1.0 ± 16.24 B	12.5
1.1 ± 49.75 A	25
1.1 ± 50.13 A	50
1.1 ± 56.08 A	75
1.1 ± 59.87 A	100

Different Letters Indicate Statistical Differences At The Level $P < 0.05$

Table (2) Showed That The Extract Aqueous Peel From *Pyrus Communis* Had An Inhibitory Effect On The Growth Of Cancer Cells Of The Hepg2 Cell Line, Starting With A Concentration Of 6.25 Mg/Ml, As The Percentage Of Inhibition Was 21.50%, And This Percentage Increased To 26.59%, 27.29% , 52.66% , 59.28% And 79.87%, For The Concentrations Used, Respectively. A Significant Difference Was Observed Between Concentrations.

Table (2) Inhibition Percentages In The Hepg2 Cell Line By The Effect Of Different Concentrations Of The Extract Aqueous Peel From *Pyrus Communis* For A Period Of 24 Hours Exposure At A Temperature Of 37 °C.

Standard Deviation. Inhibition Ratio100%	Con . Mg/Ml
1.0 ± 21.50 C	6.25
1.0 ± 26.59 C	12.5
1.1 ± 27.29 C	25
1.2 ± 52.66 B	50
1.2 ± 59.28 B	75
1.2 ± 79.87 A	100

Different Letters Indicate Statistical Differences At The Level P < 0.05

Table (3) Showed That The Extract Aqueous Peel From *Pyrus Communis* Had An Inhibitory Effect On The Growth Of Cancer Cells Of The MEF Normal Cell Line, Starting With A Concentration Of 6.25 Mg/Ml, As The Percentage Of Inhibition Was 11.20%, And This Percentage Increased To 13.24%, 20.13% , 42.75% , 55.29% And 59.87%, For The Concentrations Used, Respectively. A Significant Difference Was Observed Between Concentrations.

Table (3) Inhibition Percentages In The Control Cell Line MEF By The Effect Of Different Concentrations Of The Extract Aqueous Peel From Pyrus Communis For A Period Of 24 Hours Exposure At A Temperature Of 37 °C.

Standard Deviation. Inhibition Ratio100%	Con . Mg/Ml
1.0 ± 11.20 D	6.25
1.0 ± 13.24 D	12.5
1.1 ± 20.13 C	25
1.2 ± 42.75 B	50
1.3 ± 55.29 A	75
2.1 ± 59.87 A	100

Different Letters Indicate Statistical Differences At The Level P < 0.05

According To The Statistical Results, It Was Found That There Is A Significant Difference When Comparing The Lines MCF 7 And Hepg2 In The Extract Aqueous Peel From *Pyrus Communis*. At Concentration 100 Mg/Ml It Was Observed That The Effect Of The Aqueous Extract On The Hepg2 Line Was Greater Than That Of The MCF 7 Line. The Inhibition Percentage Was 79.87% For The Hepg2 Line And The Inhibition Percentage Was 59.87% For The MCF 7 Line, While The Inhibition Percentage For The Normal Line At The Same Concentration Was 59.87%.

Table (4) Showed That The Arbutin Peel From *Pyrus Communis* Had An Inhibitory Effect On The Growth Of Cancer Cells Of The MCF7 Cell Line, Starting With A Concentration Of 6.25 Mg/Ml, As The Percentage Of Inhibition Was 17.17%, And This Percentage Increased To 48.11 %, 49.15% , 52.59% , 59.09% And 62.12%, For The Concentrations Used, Respectively. A Significant Difference Was Observed Between Concentrations.

Table (4) Inhibition Percentages In The MCF7 Cell Line By The Effect Of Different Concentrations Of The Arbutin Peel From Pyrus Communis For A Period Of 24 Hours Exposure At A Temperature Of 37 °C.

Standard Deviation. Inhibition Ratio100%	Con . Mg/Ml
1.0 ± 17.17 C	6.25
1.0 ± 48.11 B	12.5
1.1 ± 49.15 B	25
1.1 ± 52.59 B	50
1.2 ± 59.09 A	75
1.2 ± 62.12 A	100

Different Letters Indicate Statistical Differences At The Level P < 0.05

Table (5) Showed That The Arbutin Peel From *Pyrus Communis* Had An Inhibitory Effect On The Growth Of Cancer Cells Of The Hepg2 Cell Line, Starting With A Concentration Of 6.25 Mg/Ml, As The Percentage Of Inhibition Was 26.69%, And This Percentage Increased To 39.19%, 40.10% , 62.40% , 79.39% And 92.01%, For The Concentrations Used, Respectively. A Significant Difference Was Observed Between Concentrations.

Table (4) Inhibition Percentages In The Hepg2 Cell Line By The Effect Of Different Concentrations Of The Arbutin Peel From *Pyrus Communis* For A Period Of 24 Hours Exposure At A Temperature Of 37 °C.

Standard Deviation. Inhibition Ratio100%	Con . Mg/Ml
1.0 ± 26.69 E	6.25
1.0 ± 39.19 D	12.5
1.1 ± 40.10 D	25
1.2 ± 62.40 C	50
1.2 ± 79.39 B	75
1.2 ± 92.01 A	100

Table (6) Showed That The Arbutin Peel From *Pyrus Communis* Had An Inhibitory Effect On The Growth Of Cancer Cells Of The MEF Cell Line, Starting With A Concentration Of 6.25 Mg/Ml, As The Percentage Of Inhibition Was 6.35%, And This Percentage Increased To 9.89%, 10.16% , 22.65% , 39.20% And 42.81%, For The Concentrations Used, Respectively. A Significant Difference Was Observed Between Concentrations.

Table (6) Inhibition Percentages In The Control Cell Line MEF By The Effect Of Different Concentrations Of The Arbutin Peel From Pyrus Communis For A Period Of 24 Hours Exposure At A Temperature Of 37 °C.

Standard Deviation. Inhibition Ratio100%	Con . Mg/Ml
1.0 ± 6.35 C	6.25
1.0 ± 9.89 C	12.5
1.0 ± 10.16 C	25
1.1 ± 22.65 B	50
1.1 ± 39.20 A	75
1.2 ± 42.81 A	100

Different Letters Indicate Statistical Differences At The Level $P < 0.05$

According To The Statistical Results, It Was Found That There Is A Significant Difference When Comparing The Lines MCF 7 And Hepg2 In

The Arbutin Peel From *Pyrus Communis*. At Concentration 100 Mg/Ml , It Was Observed That The Effect Of The Arbutin On The Hepg2 Line Was Greater Than That Of The MCF 7 Line. The Inhibition Percentage Was 92.01% For The Hepg2 Line And The Inhibition Percentage Was 62.12% For The MCF 7 Line, While The Inhibition Percentage For The Normal Line At The Same Concentration Was 42.81%.

Arbutin(P-Hydroxyphenyl-B-D-Glucopyranoside)Possesses Beneficial Functions Including Antioxidant, And Anti-Tumoral Activities. Due To The Important Role Of Oxidative Stress And Apoptosis In The Successful Treatment Of Cancer, Understanding Mechanisms That Lead To Apoptosis In Cancer Cells Is Essential . [12]. The Purpose Of The Current Study Was To Evaluate The Effect Of Arbutin On Tert-Butyl Hydroperoxide (T-BHP)-Induced Oxidative Stress And The Related Mechanisms In Fibroblast And Lymph Node Carcinoma Of The Prostate (Lncap) Cells. Arbutin Pre-Treatment Increased The Total Antioxidative Power And Cell Viability In The MTT Assay And Reduced *BAX/BCL-2* Expression And Necrosis In Fibroblasts ($P<0.001$). In Addition, Our Results Showed That Arbutin Can Decrease Cell Viability, Induce Apoptosis And Increase *BAX/BCL-2* Ratio In Lncap Cells At Some Specific Concentrations ($P<0.001$). **[**13**].**

Arbutin As A Potential Functional B-D-Glucopyranoside Has Strong Ability To Selectively Protect Fibroblasts Against T-BHP-Induced Cell Damage And Induce Apoptosis In Lncap Cells. [14]. .

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مستخلص البحث:

الهدف: يمتلك الأربوتين (P-Hydroxyphenyl-B-D-Glucopyranoside) وظائف مفيدة مستخرجة من نبات الكمثري *Pyrus Communis* بما في ذلك الأنشطة المضادة للأورام. نظرًا للدور الهام الذي تلعبه مضادات الورم وموت الخلايا المبرمج في العلاج الناجح للسرطان، فهو أمر ضروري. كان الغرض من الدراسة الحالية هو تقييم تأثير الأربوتين على الخط الخلوي Hepg2 وMCSF7 وMEF.

المواد وطرق العمل:

في هذه الدراسة التجريبية، تمت معالجة تأثير أربوتين والمستخلص المائي من *Pyrus Commus* في خطوط الخلايا Hepg2 وMCF7 وMEF مسبقًا باستخدام أربوتين (6.12، 12.5، 25،50، و100 ميكروغرام). بعد 24 ساعة، تمت إضافة T-BHP (30 و35 ميكروغرام) إلى الخلايا. تم قياس الجدوى (عند 24) باستخدام اختبار القياس اللوني لتقييم نشاط الخلية الاستقلابي (MTT Assay) (3-[4,5-Dimethylthiazol-2-Yl]-2,5 Diphenyl Tetrazolium Bromide)

النتائج: تم إجراء معالجة الأربوتين لفحص الخط الخلوي بما في ذلك تأثير المستخلص على نمو خطوط الخلايا السرطانية Hepg2 وMCF7 وMEF باستخدام مقايسة السمية الخلوية. أظهرت النتائج أن السمية الخلوية للمستخلص تختلف باختلاف نوع الخلية وتركيزها في خطوط الخلايا المختلفة. مع زيادة تركيزات المستخلص، زاد نشاط تثبيط خط الخلايا السرطانية. كانت أعلى نسبة تثبيط لمستخلص الاربيوتين في خط خلايا Hepg2 92.01% و 79.87 عند التركيز 100 ميكروجرام/مل للمستخلص المائي بينما في MCF7 كانت أعلى نسبة تثبيط للاربيوتين 62.12% و 59.87 في تركيز 100 ميكروجرام/مل للمستخلص المائي وكانت أعلى نسبة تثبيط لخط الخلايا MEF 42.81 .59.87% في 100 ميكروجرام/مل للمستخلص المائي.

الاستنتاج: الأربوتين كعامل وظيفي محتمل ومستخلص له قدرة قوية على التأثير المثبط لنمو الخط الخلوي وانخفاض التأثير في الخط الخلوي الطبيعي.

الكلمات المفتاحية: أربوتين؛ خط خلايا جنين الفأر الليفي؛ نبات الكمثري . خط الخلايا سرطان الكبد.