

Original Research

The medicinal plants effects on the gene expression of cytochrome P450 and P-glycoprotein in cultured colon and breast cancer cell line

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Abstract

Using herbs in the Arab world is becoming increasingly common. Hence, there is a potential for pharmacokinetic and pharmacodynamic herb-drug interactions. Cytochrome P450 (CYP) enzymes and efflux transporter such as P-glycoprotein (P-gp) are vulnerable to modulation by the multiple active constituents of herbs. To examine the effects of some herbal plants on the gene expression of CYP1A1 metabolizing enzyme and P-glycoprotein. *In vitro* approach using human breast cancer cell line MCF7 and human colon adenocarcinoma Caco2 cell line were used to evaluate the effects of ten herbal plants *Paronychia argentea* lam., *Achillea biebersteni* L., *Teucrium polium* L., *Achillea santolina* L., *Arbutus andrachne* L., *Ruta graveolens* L. *Chrysanthemum coronarium* L., *Ilex paraguariensis* A.st., *Catha edulis* Forsk., and *Anthemis palastina* Boiss on the mRNA expression of CYP1A1 and P-gp. The toxicities of these plants on the cell lines were evaluated with tetrazolium dye MTT test. Only *Catha edulis* had shown anti-proliferative activity against MCF7, but not against Caco2. *Paronychia argentea*, *Ruta graveolens* and *Catha edulis* on MCF7 and *Paronychia argentea*, *Arbutus andrachne*, *Chrysanthemum coronarium* and *Anthemis palastina* on Caco2 observed induction of CYP1A1 with dose dependant manner. Variation between induction and inhibition were noticed with plant effects on P-gp expression. *Paronychia argentea* has shown dose dependant induction effect, *Anthemis palastina* induced P-gp at high concentrations, Whereas both *Teucrium polium* and *Catha edulis* inhibit P-gp at 100µg/ml. The overall results indicate a potential food-drug interaction, which definitely should be investigated further on animal and human kinetic models.

Keywords: food–drug interaction; in-vitro; anti-proliferative effects; cytochrome P450; p-glycoprotein

INTRODUCTION

Recently, there has been a trend towards using herbal plants for therapeutic purposes. One third of adults in developing countries are using different herbal plants.¹ Therefore, the opportunity for medicinal plant–drug interaction is increasing. Perhaps most people think that all edible plants are safe and free from undesired effects. Plant-drug interaction is one of these unrevealed issues that need to be clarified for the patients and the physicians. The magnitude of the problem may appear in old poly-pharmacy (patients who are taking

many drugs) patients.

There are numerous reports of significant herb–drug interactions, and many studies have shown that these herbal constituents may induce and inhibit the drug-metabolizing enzymes. These unintended changes may result in undesired effects such as impaired bioavailability of drugs with narrow therapeutic indexes, altered drug plasma or tissue level, and enhanced bioactivation of drugs to reactive intermediates or toxics.² Cytochrome P450 (CYP450) enzymes are responsible for metabolizing endogenous components and xenobiotics. In this study, CYP1A1 was chosen because it is well characterized for its ability to metabolize xenobiotics and bioactivate many of them to carcinogens.³

P-Glycoprotein (P-gp) is the most common molecular pump protecting cells from toxic molecules. It detoxifies normal tissues from xenobiotic compounds present in herbs which are expected to be substrates of this efflux transporter⁴. Another important protein is AhR, a ligand-activated transcription factor, that regulates the transcription of a wide range of genes, including some drug-metabolizing enzymes. This receptor belongs to heterodimeric transcriptional regulators that control diversity of physiological processes such as circadian rhythms, organ development, neurogenesis, metabolism and in the stress response to hypoxia. Upon ligand binding, the receptor translocates to the nucleus and heterodimerizes with aryl hydrocarbon nuclear translocator. This heterodimer now binds to a partially characterized set of co-activators and/or co-repressors, and the resulting complex interacts with consensus

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regulatory sequences located upstream in the promoter of target genes (e.g., CYP450 such as CYP1A1).³

This study examines the effects of some herbal plants used extensively in the Arabic world on the gene expression of CYP1A1 metabolizing enzyme and P-glycoprotein, the main transporter gene of Multi-Drug Resistance Proteins (MDR1). This study used two cancer cell lines: The MCF7 breast cancer cells and Caco colon adenocarcinoma Caco2. The finding of this work will be an important indicator for the potential food-drug interactions, especially for CYP1A1 and/or MDR.

MATERIALS AND METHODS

Herbal plants collection, identification, and extraction

The wild plants—*Paronchia argentea*, *Achillea biebersteini*, *Teucrium polium*, *Achillea santolina*, *Arbutus andrachne*, *Ruta graveolens*, *Chrysanthemum coronarium*, and *Anthemis palastina* were collected from Ramtha and Houfa / Irbid at the Northern part of Jordan during April-May, 2011. *Ilex paraguariensis* was purchased from the market, and *Catha edulis* was obtained from Yemen. The plants were spread in 3 square meters shaded area for air-drying at room temperature for about two weeks. After drying, exact weights were recorded. Plant material grounded to powder using a laboratory mill. Powdered materials were maintained at room temperature (22-23°C) and protected from light until required for extraction and analysis. The ground and prepared materials were extracted with 70% ethanol, 10 gm of each plant was immersed with 100 ml of 70% ethanol for 24 hours then filtered; the residue was obtained after ethanol evaporation using rotator vacuum. The net weight of extracts were calculated and 0.1 gm of each was dissolved in 1 ml (Dimethyl sulfoxide) DMSO. The following concentrations, 200 µg/ml, 100 µg/ml, and 50 µg/ml, were used in Cell culture treatment.

Cell culture

Growth conditions

Two cell lines were used in this study MCF7 (human breast cancer cell line) and Caco2 (human colon adenocarcinoma).

Both types of cells were maintained as an attached monolayer culture in commercially defined Roswell Park Memorial Institute Medium (RPMI 1640) (HyClone, USA) for MCF7 cell lines and DMEM ((Dulbecco's Modified Eagle Medium, high glucose 4.5 g/l) (Lonza, Belgium) for Caco2 cell lines supplemented with; 10% (v/v) heat-inactivated fetal bovine serum (FBS) (HyClone, USA), 100 u/ml and 100 µg/ml penicillin-streptomycin (HyClone, USA) respectively and 2 mM L-glutamine. Cells were handled in a tissue culture incubator (Nuair, USA) at 37°C in a 90% humidified atmosphere of 5% CO₂.

Cells harvesting and counting

When the attached cells reach 80% confluency, they were trypsinized with trypsin after media aspiration and washing with phosphate buffer saline (PBS). Trypan blue was widely used for cells staining. A mixture 1:1 of Trypan blue with Cells

suspension was used in Counting. Cells were counted in specific chamber under inverted light microscope.

MTT cell proliferation assay

Antiproliferative effects of the plants on cells were evaluated by using MTT assay. This assay is a colorimetric test based on the reduction of 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), a yellow tetrazole, to a purple formazan, a process that occurs in the mitochondria of viable cells. Serial dilution of plant extracts were prepared using DMSO to prepare the following concentration 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml and 1.56 µg/ml.

Cells Treatment

To evaluate the effects of plant extracts on the gene expression of the cells, 200 µg/ml, 100 µg/ml and 50 µg/ml concentrations were used to treat 1.5×10^6 cells cultured in 25 cm² flask seeded one day before with a final DMSO concentration of 1 %. Untreated cells as well as DMSO treated cells were used as controls in the experiment. After 24 hours of treatment, the cells were harvested for mRNA extraction.

RNA isolation

Total RNA was extracted from MCF7 and Caco2 cell lines with Trizol LS reagent (Invitrogen). To Eppendorf tubes 250 µl of cells suspensions and 750 µl of Trizol were added with a final volume ratio of 1:3 followed by pipetting several times to lyse the cells. The homogenized samples were incubated for 5 minutes to permit the dissociation of nucleoprotein complexes. Then 200 µl of chloroform were added with shaking the tubes vigorously by hand for 15 second followed by incubation for 15 minutes at room temperature.

After centrifugation at 12000 xg for 15 min. Three layers were obtained. RNA remains exclusively in the upper colorless aqueous phase which was transferred to new Eppendorf tubes. To precipitate the RNA from the aqueous layer 500 µl of Isopropyl alcohol were added to the aqueous phase followed with incubation for 10 min. at room temperature. After that centrifugation at 12000 xg for 10 min. made RNA appeared as gel-like pellet. The supernatant was removed and 1 ml of 75% ethanol was added for RNA washing, followed by vortexing and centrifugation at 7500 xg for 5 min. The pellet was air-dried for 5 min and resuspended with 50 µl nuclease free water.

RNA quality was assessed by measuring RNA concentration and the ratio between RNA and protein OD RNA/ OD protein i.e. OD 260/OD 280 using a spectrophotometer (Bio-Rad, USA).

All samples concentrations were high enough to proceed into complementary single strand DNA (cDNA) synthesis and had OD 260/OD 280 ratio between 1.8-2

cDNA synthesis

1 µg of RNA from each sample was used to prepare cDNA by using (Thermo Scientific Kit). According to manufacturer protocol, RNA was diluted with nuclease free water to 12 µl then incubated at 65°C for 5 min. To diluted RNA, 4 µl of 5X reaction



buffer, 1X of ribolock RNase inhibitor (20u/μl), 2μl of 10mM dNTP Mix and 1 μl of Revert Aid MMuLV Reverse Transcriptase (200u/μl) were added. The total mixture is incubated for 60 min. at 42°C followed by 70°C for 5 min. to terminate the reaction. The final product was kept at -20 °C.

Polymerase Chain Reaction (PCR)

PCR was used to optimize the conditions of the primer used in RT-PCR. Three genes were used in this research CYP1A1, P-glycoprotein as a target genes which were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a house keeping gene. PCR 37 analysis was performed in a total volume of 50 μl with 2 μl cDNA. Kapa taq was used according to the protocol and the results were checked by gel – electrophoresis with 2% agarose gel.

The Forward and the reverse primers For CYP1A1 were ²:

5'- TCC AGA GAC AAC AGG TAA AAC A -3'

5'- AGG AAG GGC AGA GGA ATG TGA T -3'

The forward and the reverse primers For P-glycoprotein were:-

5'- TGC GAC AGG AGA TAG GCT G -3'

5'- GCC AAA ATC ACA AGG GTT A -3'

The Forward and the reverse primers For GAPDH were:-

5'- CGA CCA CTT TGT CAA GCT CA- 3'

5'- AGG GGA GAT TCA GTG TGG TG -3'

The PCR program for CYP1A1 was 94 °C for 2 min, followed by 45cycles of 94 °C for 30s, 60 °C for 30s and 72 °C for 30 s, with a final extension step of 72 °C for 5 min. For P-glycoprotein was 94°C for 2 min, followed by 30 cycles of 94°C for 15s, 58°C for 15s and 72°C for 15s, with a final extension step of 72°C for 5 min. While that for GAPDH was 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s, with a final extension step of 72 °C for 5 min.

Real-Time PCR (RT-PCR)

Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific) Kit was used to get relative expression of the encoding gene. According to the protocol 2.5 μl of cDNA was used. A standard curve was formulated by serial diluting ten folds of cDNA from untreated cells. In every experiment each tube in RT-PCR contained 12.5 Maxima SYBR Green qPCR Master mix (2X), forward and reverse primer of GAPDH (control gene) or CYP1A1 or P-gp(Target genes) in a final concentration of 0.3μM, 0.05 μl of ROX solution to optimize the experiment and this depended on the RT-PCR instrument, and nuclease free water up to 25 μl. Bio-Rad (Opticon) machine was used to carry out the experiment with the same conditions optimized by normal PCR.

RESULTS

Ten herbal plants were used in the study. 10 gm of each was extracted using rotary vacuum after incubation with 70%

ethanol for 24 hours. The percentage yields were as shown in Table 1.

No.	plants	Percentage yield of 10 gm plants
1	Paronchia argentea	7.73%
2	Achillea bibersteini	8.41%
3	Teucrium polium	8.44%
4	Achillea santolina	5.34%
5	Arbutus andrachne	14.70%
6	Ruta graveolens	7.40%
7	Chrysanthemum coronarium	4.30%
8	Ilex paraguariensis	17.66%
9	Catha edulis	8.60%
10	Anthemis palastina	9.00%

MTT results for MCF7 and caco2 cell lines

In order to identify the toxicity of plants extracts on MCF7 cell line, tetrazolium dye was used to evaluate several concentrations of plants extracts toxicity. Relative percentages of cell survival were calculated in comparison to the control 100%. It was found that some plants extract enhance cell proliferation while other inhibit cell proliferation. Cell survival percentages were approximately constant of nearly 100% with IC50 >200 ug/ml except *Catha edulis* which has shown anti-tumor and antiproliferative effect with IC50 ≥ 200ug/ml with MCF7 which affect the analysis of gene expression at this concentration.

The concentration effects of plant extracts on CYP1A1 in MCF7 cells

Relative RT-PCR was used to study the changes in gene expression after plant extracts treatment. The difference between Ct cycles of treated cells and control cells were used to get the fold changes in gene expression after normalizing it with the house keeping gene GAPDH. The difference between cycles numbers was between 1.0 – 11.0 cycles. When one cycle is the difference between treated cell and control cell, the effect of plant extract on CYP1A1 gene expression could be neglected meaning that no further investigation is recommended in comparison to that having high change in cycle number at which it cross threshold level.

The effect of different plant extracts concentrations on CYP1A1 using caco2

Relative RT-PCR was used to study the gene expression changes after plant extracts treatment. The difference between Ct cycles of treated cells and control cells were used to get the fold changes in gene expression after normalizing it with the housekeeping gene GAPDH. the difference between cycles numbers was between 1.0 – 6.0 cycle When one cycle is the difference between treated cell and control cell, the effect of plant extract on CYP1A1 gene expression could be neglected meaning that no further investigation is recommended in



comparison to that having high change in cycle number at which it cross threshold level. In comparison to MCF7 cell line, more induction was noticed in MCF7 and this show the difference between cell lines in expressing CYP1A1 gene.

The concentration effects of plant extracts on p- glycoprotein using caco2

In this study, Variation between induction and inhibition was observed in P-gp gene expression. *Paronychia argentea* has shown dose dependant induction effect while *Arbutus andrachne*, *Ruta graveolens*, and *Ilex paraguariensis* have shown constant induction response to various concentrations. Strong induction was noticed with 100 µg/ml and 200 µg/ml of *Anthemis palastina*. Several biphasic effect with different behaviors were noticed *Achillea bibersteni* induce P-gp and then inhibit it at high concentration, *Teucrium polium* induce then inhibit then induce again, *Achillea santolina* has shown strong inhibition at 50 µg/ml and 100 µg/ml, strong inhibition were noticed with 200 µg/ml of *Chrysanthemum coronarium* and 100 µg/ml *Catha edulis*.

DISCUSSION

The anti-tumor activities of plant extracts on MCF7 & Caco2 cell lines were evaluated using tetrazolium dye. The toxicity of several plants showing a concentration and time dependant cytotoxic effects were reported.⁵ With the exception of *Catha edulis* none of the plant has shown any activity against the proliferation of both the MCF7 and Caco2 cells suggesting that most of those plants are free of any cytotoxic activity and again correlate with their edible nature. Interestingly some of the plants such as *Anthemis palastina* has shown pro-proliferation activity. This pro-activity was clear on both cell lines. One explanation for these phenomena can be related to the effect of the plant extract on the AhR. Several studies have shown that xenobiotic response elements such as CYP450 mainly

CYP1A1 expression is related to AhR activation. Plants extracts contain many xenobiotics that affect AhR, A previous study⁶ has reported that AhR can act as a regulator of cell proliferation. One of the most exciting aspects of AhR biology is the ability of this receptor to promote or inhibit cell proliferation depending on cells phenotype. (Figure 1)

On the other hand, the *Catha edulis* has shown anti – proliferative activity against MCF7 cell line only. Again this effect may be explained through the action on the AhR. There are many reports demonstrating that the AhR may have an anti-proliferative activity. These reports have shown that upon activation of AhR with exogenous ligands, the AhR transcriptionally activated the tumor suppressor genes and inhibit cell proliferation.⁶ Interestingly, CYP1A1 expression is related to AhR activation and *Catha edulis* illustrated the highest up-regulation effect on the expression of CYP1A1 among the used plant at 100 ug/ml in the MCF7 cells but not on the Caco 2 cells.

One of the objectives of this study was to carry out expression analysis of xenobiotic metabolizing enzyme CYP1A1 in MCF7 cell line as *in-vitro* model after treatment with different concentrations of plants extracts. Several studies have demonstrated the positive relationship between the induction of CYP1A1 and the incidence of several human cancers such as lung, colon, and rectal cancers. Additionally, it has been demonstrated that the inhibition of AhR activity and its regulated gene, CYP1A1, could result in the prevention of toxic effects caused by the AhR ligands, including carcinogenicity.⁷

In our study, *Paronychia argentea*, *Ruta graveolens*, *Chrysanthemum coronarium* and *Catha edulis* up-regulate CYP1A1 expression in a dose dependant manner after 24 incubation with plants extract. While other plants extract show triphasic response; *Achillea bibersteni* and *Ilex paraguariensis* have shown upregulation in CYP1A1 expression followed by a decrease in induction at high concentration. In contrast,

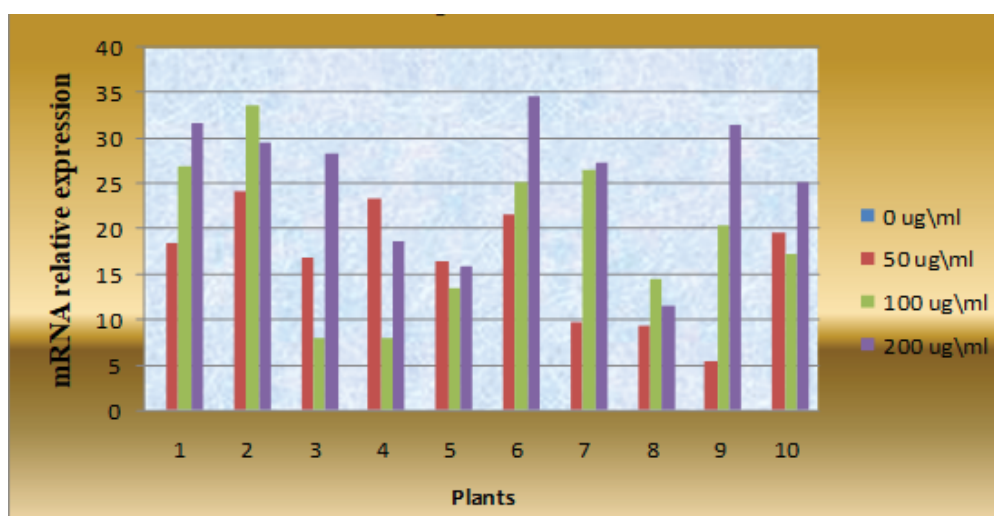


Figure 1. The effects of 0 ug/ml, 50 ug/ml, 100 ug/m, 200ug/ml of different plants extracts on the expression of CYP1A1 in MCF7 cell line. The plant extract numbering as the following: 1. Paronchia argentea 2. Achillea bibersteni 3. Teucrium polium 4. Achillea santolina 5. Arbutus andrachne 6. Ruta graveolens 7. Chrenysanthum coronanium 8. Ilex paraguariensis 9. Catha edulis 10. Anthemis palastina.

Teucrium polium, *Achillea santolina*, *Arbutus andrachne* and *Anthemis palastina* have shown induction level at 100ug/ml less than that observed with 50ug/ml and 200 ug/ml. AhR receptor was known as (dioxin) receptor because it mediate most of the toxic and carcinogenic effects of a wide variety of environmental contaminants such as dioxin (TCDD; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin).⁷ Several other compounds were known to induce CYP1A1 such as b-naphthoflavone and 3- methylcholanthrene. It was found that after mRNA and protein level analysis that different patterns of effects could be present by acting differently on transcription and translation⁸ Moreover, time and concentration dependant modulation of TCDD induced CYP1A1gene expression were found.⁹

Flavonoids are important constituents of human diet including fruits, vegetables and herbal plants. It was reported that these compounds have modulatory effects on the expression of several genes and most likely through AhR pathway.⁹ Two recent hypothesis concerning flavonoids - polyphenols and CYP1A1 have been established. Based on the structural diversity of each polyphenolic moiety, in terms of hydroxyl or methoxy substitutions, certain flavonoids with multiple hydroxyl groups are considered more effective CYP1A1 inhibitors, whereas flavonoids with multiple methoxy groups are thought to exhibit higher rate of CYP1A1 metabolism.¹⁰

Flavonoids which mainly present in several plants extracts and responsible for plants effects are structurally classified into eight groups: flavans, flavanones, isoflavanones, flavones, isoflavones, anthocyanidines, chalcones, and flavonolignans. Many flavonoids have been reported to be potent inducers of various CYPs For example, galangin, quercetin, genistein, diosmin, and its aglycone form, diosmetin, increased the expression of CYP1A1. (Figure 2)

According to a previous study,¹¹ quercetin and genistein induce CYP1A1 in a dose dependant manner However, the inhibition of gene expression of CYP1 by flavonoids was also

observed. For example, quercetin binds as an antagonist to AhR receptor, and consequently inhibits CYP1A1 by decreasing mRNA accumulation when incubated with TCDD.¹¹ Moreover, dimethoxyflavone and Chrysin other flavonoids compound has shown inhibitory effects on CYP1A1.

In our study, *Paronychia argentea*, *Ruta graveolens* and *Catha edulis* have been the most potent inducer for CYP1A1. Literature has shown that isoflavone (genistein) is one of the main constituents in *Paronychia argentea* and flavonl (quercetin) is one of the main constituents in *Catha edulis* and *Ruta graveolens*. We suggest that these constituents might be the cause of CYP1A1 induction and variation in CYP1A1 expression depends on the main constituents of the plants extracts and variation in response between dose dependant effects and biphasic effects could be explained by the several constituents in herbal plant which dominates differently at different concentrations.¹²

Modulation of CYP1A1 could be clinically important. CYP1A1 and CYP1A2 have been implicated in increased carcinogenic activation of chemicals. Thus, they are considered as a potential risk factor in certain cancers and hence drugs that induce these reactions are preferentially avoided by the pharmaceutical industry. (Figure 3)

Several plants extracts have shown effects on CYP1A2, which is controlled through AhR, for example, *G.biloba* has shown biphasic inhibition of CYP1A2 begin with induction at low concentration followed with inhibition at high concentration. This is clinically important when it is taken with omeprazole, clozapine and theophyllin a typical CYP1A2 substrate. Since CYP1A1 as well as CYP1A2 are modulated by AhR, such interactions might be daily occurrence and variation in response between dose dependant effects and biphasic effects could be explained by that herb contains several constituents and the effects of these different constituents dominates differently at different concentrations

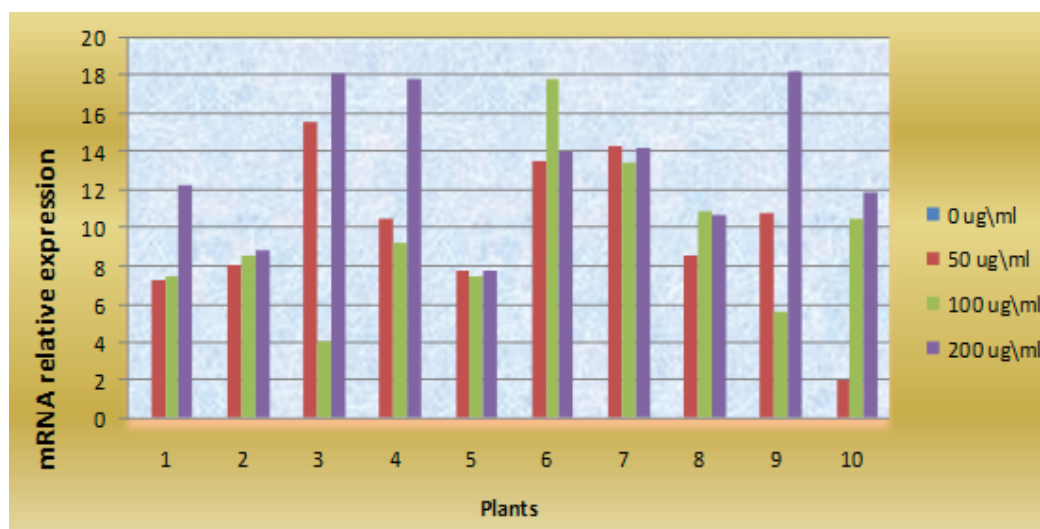


Figure 2. The effects of 0 ug/ml, 50 ug/ml, 100 ug/m, 200ug/ml of different plants extracts on the expression of CYP1A1 in Caco 2 cell line. The plant extract numbering as the following: 1. Paronchia argentea 2. Achillea bibersteni 3. Teucrium polium 4. Achillea santolina 5. Arbutus andrachne 6. Ruta graveolens 7. Chrenysanthum coronanium 8. Ilex paraguariensis 9. Catha edulis 10. Anthemis palastina.



Figure 3. The effects of 0 ug/ml, 50 ug/ml, 100 ug/m, 200ug/ml of different plants extracts on the expression of P-gp in Caco 2 cell line. The plant extract numbering as the following:1. *Paronychia argentea* 2. *Achillea bibersteni* 3. *Teucrium polium* 4. *Achillea santolina* 5. *Arbutus andrachne* 6. *Ruta graveolens* 7. *Chrenysanthum coronanium* 8. *Ilex paraguariensis* 9. *Catha edulis* 10. *Anthemis palastina*.

Plants extract effects on CYP1A1 using Caco2 cell line: Epithelial cells of the gastrointestinal tract are responsible for the resorption of food components and may also influence the resorption of xenobiotics. It is equipped with a variety of enzymes and efflux pumps that are able to metabolize the entry components. Caco2 cells were used for the *in vitro* investigation of herbal extracts on the expression of CYP1A1 because these cells are known to display the morphological and characteristics of human enterocytes, furthermore it expresses multiple enzyme including CYP1A1.¹³

In our study, *Paronychia argentea*, *Achillea bibersteni*, *Arbutus andrachne* *Chrysanthemum coronarium* and *Anthemis palastina* up-regulate CYP1A1 expression in a dose dependant manner after 24 incubation with plants extract. While other plants extract show triphasic response; *Ruta graveolens* and *Ilex paraguariensis* have shown upregulation in CYP1A1 expression followed with decrease in induction at high concentration. In contrast, *Teucrium polium*, *Achillea santolina*, and *Catha edulis* have shown decrease in induction at 100ug/ml in comparison with the induction at 50ug/ml and 200 ug/ml.

In correlation to MCF7, the same behavior of dose dependant induction was observed with *Paronychia argentea*, *Chrysanthemum coronarium* and the same triphasic effect with *Teucrium polium*, *Achillea santolina* and *Ilex paraguariensis*. It's worth mentioning that *Teucrium polium* cause less induction at 100µg/ml than that at 50µg/ml and 200 µg/ml in both MCF7 and caco2 in a way emphasizes the need for further investigation to explain this phenomenon to know the main compound responsible for this effect.

Since enterocytes are the first major site of metabolism, modulation of enzyme activity were studied. CYP1A1 activity at the human intestinal level would be of primary importance and it's well studied. Moreover, Modulation of CYP1A1 by AhR was well reported in caco2 cell line.¹⁴

Level of induction in caco2 is less than that in MCF7, this might be because enterocytes are normally exposed to several xenobiotics and so it normally expresses higher level of

CYP1A1. This what was observed by comparing control cells of MCF7 and Caco2 using RT-PCR so a little induction is needed for xenobiotics exposure.

Several toxic substances enter the body and attack the molecular machinery of several enzymes. The body either detoxify them by certain enzymes or eject them out side by certain efflux pumps such as P-gp. Since the intestine is the main site of absorption, preclinical prediction of drug absorption is a continuous challenge in drug development since passive diffusion and active efflux pumps makes the process complex. The complexity of the process increases when the drug is taken with herbal plant that modulate the efflux pumps and affect the bioavailability of the drugs. Several clinical evidences had postulated the effects of plants extract on P-gp efflux pumps such as STW]] and Garlic which induce P-gp affecting the bioavailability of several drugs such as Cyclosporine, Saquinavir, oral contraceptive, digoxin, verapamil and so forth.

In this study, Variation between induction and inhibition was observed in P-gp gene expression. *Paronychia argentea* has shown dose dependant induction effect while *Arbutus andrachne*, *Ruta graveolens*, and *Ilex paraguariensis* have shown constant induction response to various concentrations. Strong induction was noticed with 100 µg/ml and 200 µg/ml of *Anthemis palastina*. Several biphasic effect with different behaviors were noticed *Achillea bibersteni* induce P-gp and then inhibit it at high concentration, *Teucrium polium* induce then inhibit then induce again, *Achillea santolina* has shown strong inhibition at 50 µg/ml and 100 µg/ml, strong inhibition were noticed with 200 µg/ml of *Chrysanthemum coronarium* and 100 µg/ml *Catha edulis*.

The variation in response caused by the several constituents present in plants extracts which dominates differently at different concentrations which might modulate P-gp in different ways. Moreover, specific and high affinity binding of natural molecule to p-gp is a matter of discussion since investigation suggested multiple different binding sites, meaning that the molecules might bind to more than one binding domain depending on their structural properties.⁴

Several natural compounds were reported to have effects on P-gp such as flavonoids alkaloids, etc. each compound of plant extract acts differently from the other. For example; It was reported that Quercetin and genistein induce P-gp leading to decrease the bioavailability of several drugs.^{4,15}

CONCLUSION

In our study, *the presence of genistein could explain Paronychia argentea dose-dependant manner of induction. Ruta graveolens* induction effect might be a cause of quercetin. p-gp displays a remarkable ability to interact with a large variety of compounds. Further investigation with theses herbal extracts help in prediction the possible interactions that might affect

the previous mentioned drugs.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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Include any sources of funding of your study if applicable or state "Authors received no specific funding for this work".

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