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Effects of Natural Products on ABCB1 Transporter in Rat Everted Gut

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Abstract

ATP-binding Cassette (ABC) efflux transporters affect many of drug absorption. These efflux transporters limit the availability of a wide range of substances and especially cancer treatment medications. The effect of Curcumin and Panax ginseng on ABCB1 efflux activity was determined by measuring two ABCB1 substrates, verapamil and tamoxifen permeability in rat everted gut.

The concentrations of absorbed verapamil and tamoxifen were measured by HPLC in the existence or not of Curcumin and Panax ginseng at variable time points (5, 15, 30, 45 and 60 min) by everted gut sacs model.

At 60 min incubation, the permeability of verapamil in presence of tamoxifen, Curcumin and Panax ginseng was significantly ($p \leq 0.001$) increased by 2, 1.8 and 1.6- fold, respectively. Additionally, the concentration of tamoxifen that passed through the intestine wall elevated significantly ($p \leq 0.001$) when it was incubated with verapamil Curcumin and Panax ginseng by 1.8, 1.6 and 1.5-fold, respectively.

This is the first study that established the supressing effect of Curcumin and Panax ginseng on gastrointestinal tract -ABCB1 transporter activity.

Keywords: *P-glycoprotein, anticancer drugs, everted gut, Curcumin and Panax ginseng.*

Introduction

ATP-binding cassette (ABC) transporters are considered as a huge transporters family which efflux transports many substrates through extracellular and intracellular membranes, such as peptides, ions, ,sterols, metabolic constituents, lipids, toxins and medical drugs [1, 2]. The ABC transporters that are expressed intensively in intestinal epithelial cells are ABCB1 (p-glycoprotein), ABCG2 (Brest cancer resistant protein) and ABCC1 (Multidrug Resistance Protein) in addition to other members that are expressed in less intense [3, 4].

Some members of the ABC transporters have a major activity in the process of multidrug resistance (MDR), for instance patients that are getting an anticancer regime can develop resistance not only to the cancer treatment regime drugs they getting but also to several other kinds of drugs [5]. Furthermore to deliberating MDR in cancer cells, ABC transporters reduce the transportation of numerous drugs from GIT tract, and transfer drugs between the liver and the biliary as way of getting rid of external constituents out of the body [5]. As follows, a huge number of substrates that are transferred by ABC

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transporters or get effect on the transfer of many other management medications thus changing the bioavailability of these medications [6]. Currently, the approach to increase permeability through the intestine has focused towards targeting drugs that pass through the intestinal epithelial membrane transporters [7].

Numerous natural products are identified for their therapeutic properties and their ability to effect on ABC transporters activity [8]. Inhibition of ABCB1 by natural products is considered as an advanced method for reversing chemotherapies drug resistant avoiding any undesirable toxic effects [9].

Curcumin is present in nature as polyphenol normally presents in Curcuma longa (turmeric). it is traditionally well-known as anti-cancer, anti-oxidant, anti-arthritic and anti-inflammatory properties [10, 11]. It known to reduce, and reduce cancer proliferation at every phase of the disease [12]. The anti-tumors activity of tumeric have been first of all related to its action of blocking factor-kappa B (NF-kappa B) that mediates cell proliferation ,inflammation and death in normal cells [13].

Recently, studies have approved that tumeric inhibits the action of some ABC drug transporters (ABCB1, ABCG2 and ABCC1) [14, 15]. Based on study results, turmeric can avoid cancer drug resistance caused by ABC transporters. Moreover, it also increase the availability of tumor drugs which have poor intestinal absorption because of the active efflux by ABC transporters [16].

Ginseng is a common herbal drug for very long time. The major constituents of ginseng are ginsenosides. Up to this day, about 40 ginsenosides types have been identified and characterised [17], and discovered to have many pharmacological actions that involved anti-oxidant , anti-inflammatory, anti-tumer and immune modulation effects [18-20]. Ginsenosides able to inhibit ABCB1 transporter activity and so improve the availability of drugs like anticancers that are a substrate to this efflux transport [21].

In the present study the effects of Curcumin and Ginseng on the activities of ABCB1 and on the permeability of verapamil and anti-cancer drug tamoxifen were investigated in a well-characterised intestinal everted gut sacs model.

Experimental

Materials

Chemicals and drugs were purchased from Sigma-Aldrich. Curcumin and panax gensing were purchased from now foods (U.S.A).

Other chemical reagents used in this study were of analytical grade.

Preparation of rat everted sacs

The everted sac procedure was used as described in literature [22, 23]. All rats experiments were permitted by the local ethics committee of the University of Baghdad (Baghdad, Iraq). Male Sprague–Dawley rats with weighing 200 and 260 g were fasted for at least 24 h and were allowed for free access to water. Rat under anaesthesia condition , the jejunum of the intestines was excised; the segment was washed several times with normal saline solution (0.9% NaCl) and kept in oxygenated Ringer buffer solution. Ringer solution composed of 150 NaCl, 5 KCl, 1 MgCl2, 2 CaCl2, and 10 mM glucose. The intestines were everted on a glass rod; and filled with oxygenated buffer .Then; each one was divided into sacs of 3.5-4 cm length by using silk sutures. The sacs were kept in the oxygenated buffer solution, the solution was retained with 95% O2 and 5% CO2 at 37 °C during the experiment duration.

Drug ABCB1-transporter study

Sacs were transferred into 25 mL of oxygenated ringer solutions containing verapamil (100 μM) alone (control), tamoxifen (10 μM) alone (control), verapamil and tamoxifen together, verapamil or tamoxifen with curcumin (10 μM) or Panax ginseng (200 μM).

At various times points 0, 5, 15, 30, 45 and 60 min, sacs were removed, washed many times in normal saline and the serosal contents were collected. Samples stored in the refrigerator until analysis.

Verapamil and Tamoxifen concentrations were measured by a validated HPLC method. The weight of all sacs was recorded at the beginning and after the experiment to determine the fluid volume inside each sac.

Assessment of everted gut sacs viability

The viability of the gut sac was studied by measuring the glucose concentrations both inside and outside the sac, glucose level was measured by a monitor (One Touch, UK). The cell damage was examined by determining the release of lactic dehydrogenase enzyme (LDH), it measured by using LDH kit by scientific laboratory (Iraq). The results were considered as U/L/cm2 of sac area.

Samples analysis by HPLC

Verapamil and tamoxifen concentrations were measured by a HPLC assay.

For verapamil samples, the HPLC system (Thermo Separation Products, USA) was set at a wavelength of 200 nm run with C18 column (4.6mm×150mm, 5m, Waters Co., Milford, MA, USA) with a flow rate of 1 mL/min. Mobile phase contained 40% acetonitrile and 0.025 mol/L KH2PO4 with pH 2.5 [24].

For tamoxifen sample, the HPLC system was set at a wavelength of 254 nm with an emission cut-off filter of 360 nm and coupled to the same column mentioned above and with a flow rate of 1 mL/min. Mobile phase contained 20 mM dipotassium hydrogen phosphate (pH 3.0, adjusted with phosphoric acid)-acetonitrile (60:40, v/v) [25].

All measured of verapamil and tamoxifen samples concentrations were above the lower limit of quantification.

Statistical analysis

All tests were done four times. Data are measured as mean ± standard deviation and statistical calculations were carried out by using SPSS v20 (IBM) to perform unpaired t-tests. A P-value of

≤ 0.05 is take into consideration as significant.

Results

Everted gut viability

The viability of everted gut sacs were determined by measuring glucose concentration in both sides, in serosal fluid and mucosal fluid.

During the experiments and at 60 min, the glucose concentration in the serosal fluid (outside the sac) was about 1.6 fold more than the mucosal (inside the sac) concentration at 60 min. These data evidently prove that the glucose absorption is active and the gut sacs were physically intact and active through the whole experiments duration.

Additionally, LDH enzyme activity was measured as an additional marker for sacs viability. The mean of LDH activity measured in the incubation buffer was around 140 ± 7 U/L/cm2

At 15, 30 and 60 min, there was no significant differences between the time points showing the viability of the gut sacs throughout the experiments duration.

Effect of natural products on ABCB1 activity

Effect of natural products on verapamil absorption

Studies were carried out to determine the effect of Curcumin and Panax ginseng exposure on verapamil transport by ABCB1 through the small intestine wall.

The permeability of verapamil in presence of tamoxifen, Curcumin and Panax ginseng increased in time dependent manner at 5,15, 30 min until it reach to plateau situation at 45 min and 60 min (Figure 1).

After 60 min incubation of gut sacs with ABCB1 inhibitor tamoxifen (10 µM), the verapamil permeability increased significantly by 2-fold ($P < 0.01$) compared to verapamil alone (control). Incubation of gut sac with Curcumin and Panax ginseng at concentration of 10 µM significantly increase verapamil permeability by 1.8-fold and 1.6 –fold, respectively compared with control ($P < 0.01$) (Figure 2).

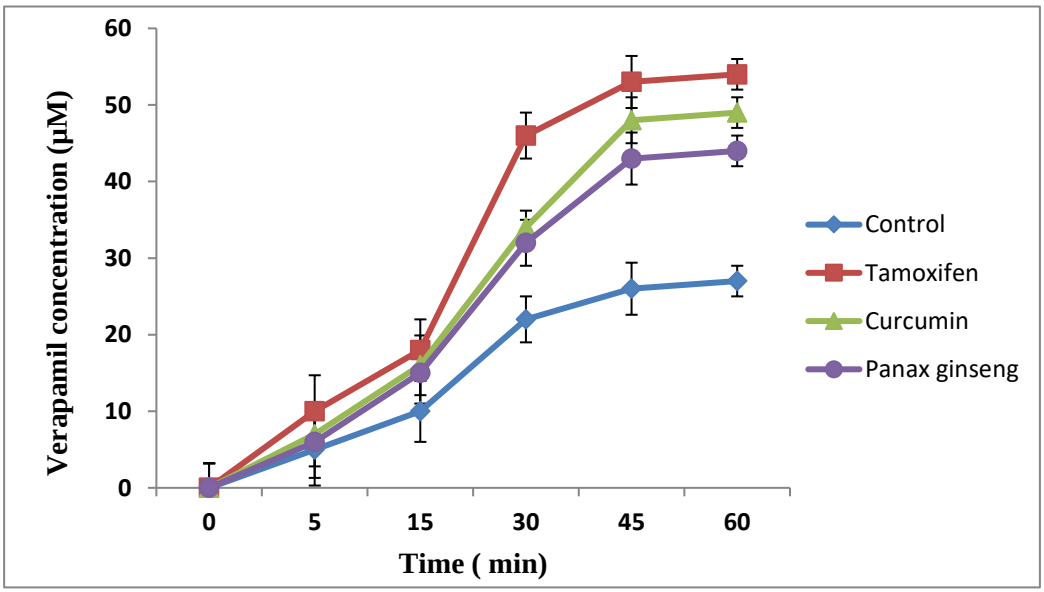

Figure 1. The absorption curve of verapamil versus verapamil–inhibitors in everted rat intestinal sac.

The absorption curve of verapamil (100 µM) in the everted rat intestinal sac versus time after 5, 15, 30, 45 and 60 min incubation, and the following: (♦) control (buffer) (■) with Tamoxifen (10 µM), (▲) Curcumin (10 µM), (■) Panax ginseng (200 µM).

Data were analysed using unpaired Student's t-test and are presented as the mean ± SD of 4 independent experiments. ** $p \leq 0.001$.

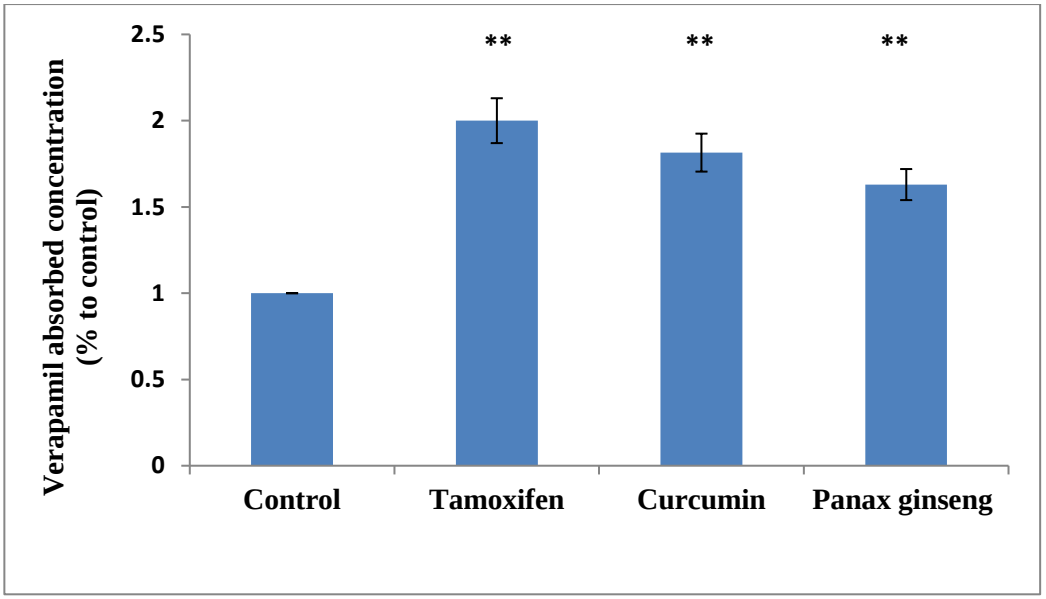

Figure 2. The effect of ABCB1 inhibitors on verapamil permeability in everted rat intestinal sac at 60 min incubation.

Data were analysed using unpaired Student's t-test and are presented as the mean ± SD of 4 independent experiments. ** $p \leq 0.001$.

Effect of natural products on Tamoxifen absorption

Further Studies were carried out to examine the effect of Curcumin and Panax ginseng exposure on tamoxifen (Breast cancer medication) absorption through the small intestine wall.

Incubation of intestinal sacs with tamoxifen showed a low absorption rate due to efflux mechanism of ABC transports. The permeability of tamoxifen through the gut sac significantly increased in presence of verapamil, Curcumin and Panax ginseng, and this elevation was increased with the time (Figure 3).

The tamoxifen permeability significantly improved after 60 min incubation of gut sacs with ABCB1 inhibitor verapamil (100 µM), its concentration increased by 1.8-fold ($P < 0.01$) compared to tamoxifen alone (control) (Figure 4). However, incubation of gut sac with Curcumin and Panax ginseng (10 µM) significantly increase tamoxifen permeability by 1.6-fold and 1.5 –fold, respectively compared with control ($P < 0.01$) (Figure 4).

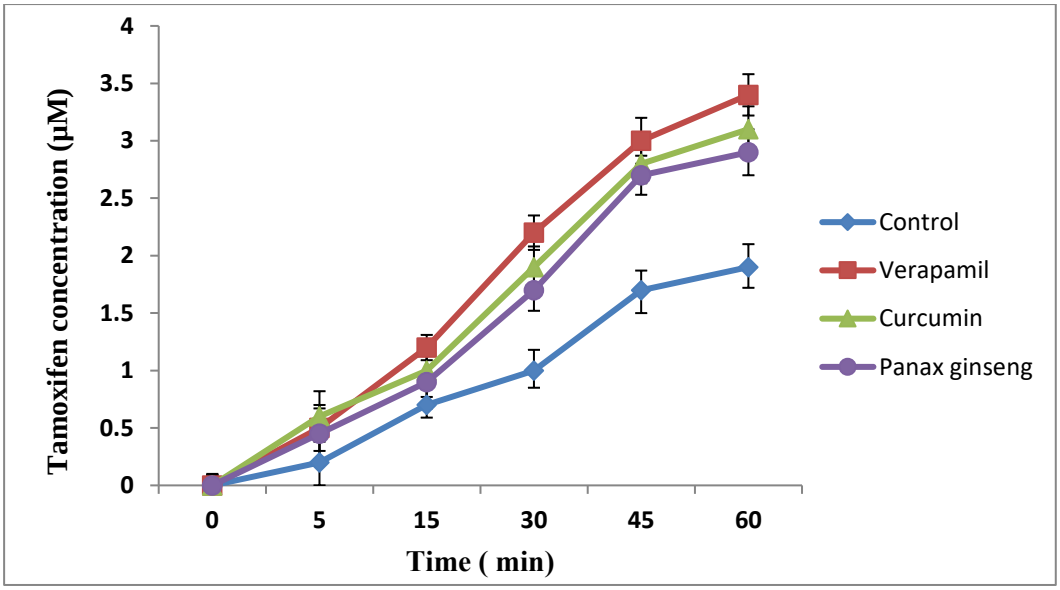

Figure 3. The absorption curve of tamoxifen versus tamoxifen –inhibitors in everted rat intestinal sac.

The absorption curve of Tamoxifen (10 µM) in the everted rat intestinal sac versus time after 5, 15, 30, 45 and 60 min incubation, and the following: (♦) control (buffer) (■) with verapamil (100 µM), (▲) Curcumin (10 µM), (■) Panax ginseng (200 µM).

Data were analysed using unpaired Student's t-test and are presented as the mean ± SD of 4 independent experiments. ** p≤0.001.

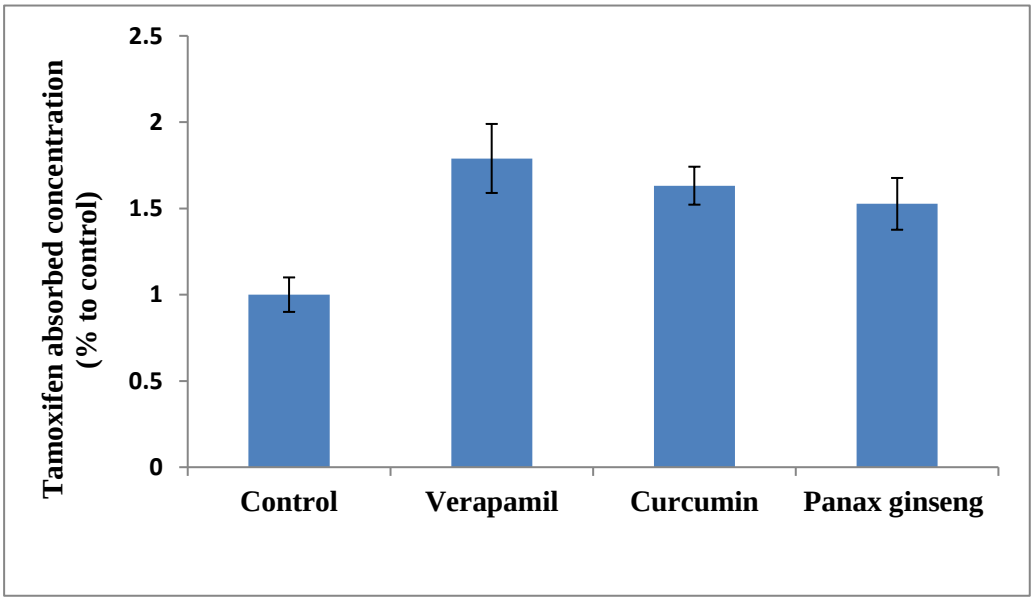

Figure 4. The effect of ABCB1 inhibitors on tamoxifen permeability in everted rat intestinal sac at 60 min incubation.

Data were analysed using unpaired Student's t-test and are presented as the mean ± SD of 4 independent experiments. ** p≤0.001.

Discussion

ATP-binding cassette (ABC) transporters are active transmembrane proteins that are broadly expressed in a many membranes of tissues and organs [26]. The intestinal epithelial membrane intensively expresses ABC transporters such as ABCB1, ABCC and

ABCG2 , these ABC transporters affect the oral bioavailability of wide range of drugs such as chemotherapy agents [27].

Tamoxifen is a nonsteroidal antiestrogen drug and is the drug of choice for treating breast cancer. Due to its low toxicity, it became widely used as a chemotherapeutic agent [28]. Tamoxifen is a substrate for the efflux of ABCB1, breast cancer resistance protein (ABCG2) and other members of ATP-binding cassette (ABC) superfamily, which lead to reduce its penetration and so decrease its therapeutic effect in cancer treatment [29, 30].

In this study, we used an in vitro model of small intestine, involved of intestinal everted gut sac, to investigate the effects of natural products and ABCB1 inhibitors on ABC-transporters activity.

Characterisation experiments were carried out to check the viability and the absence of any damage in the intestinal gut sacs. Glucose is mainly absorbed by active transported in the small intestine of most animals and human, thus a glucose grade between the fluids inside and outside gut sacs is considered as a further indicator for gut function [31]. The results showed glucose concentration in the serosal side is significantly higher than the mucosal side indicating that the gut sacs were healthy and glucose was transported actively.

Furthermore, LDH enzyme activity , indicator of cell damage [23], was steady at different time points until 60 min, the end of the experiments. This finding support that there was no damage in gut sacs until during the incubation time. These finding is in agreement with literature studies [22, 32].

This study focused on using natural herbs to increase the absorption and bioavailibity of drugs that suffer from low absorption due to ABC efflux transport. Verapamil is a widely used as an ABCB1 substrate and as a standard inhibitor to study the inhibitory effect of other substances [33, 34]. Tamoxifen is a very common agent used for cancer treatment [35, 36], and it is an ABCB1 transporter substrate which lead to low absorption through the intestine [37].

Initial studies were carried out to confirm the functional activities of ABCB1. Our results of incubation of intestinal everted sac with tamoxifen (ABCB1 inhibitor) significantly elevates verapamil permeability (2-fold). Additionally, in lateral experiments, verapamil increased tamoxifen penetration by 1.8- fold ($p \leq 0.001$). A study showed that verapamil and tamoxifen inhibit the activity of ABCB1 and increase substrates availability in cancer cell [38]. Another in vivo study support our finding, their results displayed that the plasma level of tamoxifen significantly raised by combination of verapamil oral administration by range 1.6-2- fold in rats [39]. As far as we know, there was no study examined the effect of verapamil and tamoxifen combination on each other permeability extent by using intestinal everted gut sac model.

Subsequently, this study investigated the effects of two natural products, Curcumin and Panax ginseng on both verapamil and tamoxifen permeability. Curcumin and Panax ginseng both significantly decreased ABCB1 transporter activity by improving verapamil penetration from serosal side to mucosal side. This finding is supported by a study that used the MRP1-HEK 293 cell lines, the results showed that Tetrahydro-curcumin reduce ABCB1 activity by reducing its substrates concentration (vinblastine and mitoxantrone) [40]. Panax ginseng ABCB1 inhibitory effects is in agreement with a scientific study which reported Ginsenoside F1 has an inhibitory activity on ABCB1 in MDR1-MDCKII and Caco-2 cells [41].

We consequently examined the ability of Curcumin and Panax ginseng to enhance the absorption of, ABCB1 substrate and the chemotherapy agent, tamoxifen. The results reported that the above natural products significantly enhanced tamoxifen permeability suggesting the inhibitory effect of them on ABCB1 as they inhibit the efflux of verapamil

as explained above. These results are consistent with the study approved that Ginsenoside F1 inhibits ABCB1 doxorubicin-resistant in acute myelogenous leukemia sublines [42].

This is the first study to demonstrate the effect of these natural products on ABCB1 activity in gastrointestinal tract by using everted gut model.

Conclusion

The findings of the current study suggest that using of natural product could be a very useful way to improve the absorption of many medications including chemotherapy drugs which are ABC transporters substrate, especially with safe and common natural herbs. Other natural product with low toxicity could be examined in the future to improve drugs bioavailability.

Conflict of interest:

The authors declare no conflict of interest.

References

[1] A. Sajid, S. Lusvarghi, S.V. Ambudkar. THE P-GLYCOPROTEIN MULTIDRUG TRANSPORTER. Drug Transporters: Molecular Characterization and Role in Drug Disposition (2022) 199-211.

[2] M.H. Shubbar, J.I. Penny. Therapeutic drugs modulate ATP-Binding cassette transporter-mediated transport of amyloid beta (1–42) in brain microvascular endothelial cells. European journal of pharmacology 874 (2020) 173009.

[3] S. Berggren, C. Gall, N. Wollnitz, M. Ekelund, U. Karlbom, J. Hoogstraate, D. Schrenk, H. Lennernäs. Gene and protein expression of P-glycoprotein, MRP1, MRP2, and CYP3A4 in the small and large human intestine. Molecular pharmaceutics 4 (2007) 252-257.

[4] Z.M. Al-Majdoub, B. Achour, N. Couto, M. Howard, Y. Elmorsi, D. Scotcher, S. Alrubia, E. El-Khateeb, A.M. Vasilogianni, N. Alohali. Mass spectrometry-based abundance atlas of ABC transporters in human liver, gut, kidney, brain and skin. FEBS letters 594 (2020) 4134-4150.

[5] K.M. Hanssen, M. Haber, J.I. Fletcher. Targeting multidrug resistance-associated protein 1 (MRP1)-expressing cancers: Beyond pharmacological inhibition. Drug Resistance Updates (2021) 100795.

[6] P. Bugde, R. Biswas, F. Merien, J. Lu, D.-X. Liu, M. Chen, S. Zhou, Y. Li. The therapeutic potential of targeting ABC transporters to combat multi-drug resistance. Expert opinion on therapeutic targets 21 (2017) 511-530.

[7] R. Pangeni, S. Kang, S.K. Jha, L. Subedi, J.W. Park. Intestinal membrane transporter-mediated approaches to improve oral drug delivery. Journal of Pharmaceutical Investigation 51 (2021) 137-158.

[8] D. Lopez, S. Martinez-Luis. Marine natural products with P-glycoprotein inhibitor properties. Marine drugs 12 (2014) 525-546.

[9] S.-F. Zhou, Z.-W. Zhou, C.-G. Li, X. Chen, X. Yu, C.C. Xue, A. Herington. Identification of drugs that interact with herbs in drug development. Drug discovery today 12 (2007) 664-673.

[10] J.M. Witkin, X. Li. Curcumin, an active constiuent of the ancient medicinal herb Curcuma longa L.: some uses and the establishment and biological basis of medical efficacy. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders) 12 (2013) 487-497.

[11] M.C. Fadus, C. Lau, J. Bikhchandani, H.T. Lynch. Curcumin: An age-old anti-inflammatory and anti-neoplastic agent. Journal of traditional and complementary medicine 7 (2017) 339-346.

[12] V. Zoi, V. Galani, G.D. Lianos, S. Voulgaris, A.P. Kyritsis, G.A. Alexiou. The Role of Curcumin in Cancer Treatment. Biomedicines 9 (2021) 1086.

[13] H.J. Kang, S.H. Lee, J.E. Price, L.S. Kim. Curcumin suppresses the paclitaxel-induced nuclear factor-κB in breast cancer cells and potentiates the growth inhibitory effect of paclitaxel in a breast cancer nude mice model. The breast journal 15 (2009) 223-229.

[14] W. Chearwae, S. Shukla, P. Limtrakul, S.V. Ambudkar. Modulation of the function of the multidrug resistance–linked ATP-binding cassette transporter ABCG2 by the cancer chemopreventive agent curcumin. Molecular cancer therapeutics 5 (2006) 1995-2006.

[15] P. Limtrakul, W. Chearwae, S. Shukla, C. Phisalphong, S.V. Ambudkar. Modulation of function of three ABC drug transporters, P-glycoprotein (ABCB1), mitoxantrone resistance protein (ABCG2) and multidrug resistance protein 1 (ABCC1) by tetrahydrocurcumin, a major metabolite of curcumin. Molecular and cellular biochemistry 296 (2007) 85-95.

[16] W.-D. Lu, Y. Qin, C. Yang, L. Li. Effect of curcumin on human colon cancer multidrug resistance in vitro and in vivo. Clinics 68 (2013) 694-701.

[17] Y.-L. Dai, M.-D. Qiao, P. Yu, F. Zheng, H. Yue, S.-Y. Liu. Comparing eight types of ginsenosides in ginseng of different plant ages and regions using RRLC-Q-TOF MS/MS. Journal of Ginseng Research 44 (2020) 205-214.

[18] C.-f. Chen, W.-f. Chiou, J.-t. Zhang. Comparison of the pharmacological effects of Panax ginseng and Panax quinquefolium. Acta Pharmacologica Sinica 29 (2008) 1103-1108.

[19] C. Mancuso, R. Santangelo. Panax ginseng and Panax quinquefolius: From pharmacology to toxicology. Food and Chemical Toxicology 107 (2017) 362-372.

[20] S.H. Hyun, K.D. Bhilare, G. In, C.-K. Park, J.-H. Kim. Effects of Panax ginseng and ginsenosides on oxidative stress and cardiovascular diseases: pharmacological and therapeutic roles. Journal of Ginseng Research (2021).

[21] J.K. Bae, Y.-J. Kim, H.-S. Chae, D.Y. Kim, H.S. Choi, Y.-W. Chin, Y.H. Choi. Korean red ginseng extract enhances paclitaxel distribution to mammary tumors and its oral bioavailability by P-glycoprotein inhibition. Xenobiotica 47 (2017) 450-459.

[22] K.L. Hamilton, A.G. Butt. Glucose transport into everted sacs of the small intestine of mice. Advances in physiology education 37 (2013) 415-426.

[23] L. Barthe, J. Woodley, G. Houin. Gastrointestinal absorption of drugs: methods and studies. Fundamental & clinical pharmacology 13 (1999) 154-168.

[24] V. Ivanova, D. Zendelovska, M. Stefova, T. Stafilov. HPLC method for determination of verapamil in human plasma after solid-phase extraction. Journal of Biochemical and Biophysical Methods 70 (2008) 1297-1303.

[25] K.M. Fried, I.W. Wainer. Direct determination of tamoxifen and its four major metabolites in plasma using coupled column high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications 655 (1994) 261-268.

[26] D.M. Mutch, P. Anderle, M. Fiaux, R. Mansourian, K. Vidal, W. Wahli, G. Williamson, M.-A. Roberts. Regional variations in ABC transporter expression along the mouse intestinal tract. Physiological Genomics 17 (2004) 11-20.

[27] T.G. Tucker, A.M. Milne, S. Fournel-Gigleux, K.S. Fenner, M.W. Coughtrie. Absolute immunoquantification of the expression of ABC transporters P-glycoprotein, breast cancer resistance protein and multidrug resistance-associated protein 2 in human liver and duodenum. Biochemical pharmacology 83 (2012) 279-285.

[28] M. Lazzeroni, D. Serrano, B.K. Dunn, B.M. Heckman-Stoddard, O. Lee, S. Khan, A. Decensi. Oral low dose and topical tamoxifen for breast cancer prevention: modern approaches for an old drug. Breast Cancer Research 14 (2012) 1-11.

[29] K. Kiyotani, T. Mushiroda, Y. Nakamura, H. Zembutsu. Pharmacogenomics of tamoxifen: roles of drug metabolizing enzymes and transporters. Drug metabolism and pharmacokinetics (2011) 1110240239-1110240239.

[30] N.S. Alamolhodaei, H. Rashidpour, M.E. Gharaee, J. Behravan, F. Mosaffa. Overexpression of ABCC2 and NF-Kβ/p65 with Reduction in Cisplatin and 4OH-Tamoxifen Sensitivity in MCF-7 Breast Cancer Cells: The Influence of TNF-α. Pharmaceutical Sciences 26 (2020) 150-158.

[31] M. Li, L. Si, H. Pan, A.K. Rabba, F. Yan, J. Qiu, G. Li. Excipients enhance intestinal absorption of ganciclovir by P-gp inhibition: assessed in vitro by everted gut sac and in situ by improved intestinal perfusion. International journal of pharmaceutics 403 (2011) 37-45.

[32] M. Yaghoobian, A. Haeri, N. Bolourchian, S. Shahhosseini, S. Dadashzadeh. An investigation into the role of P-glycoprotein in the intestinal absorption of repaglinide: assessed by everted gut sac and Caco-2 cell line. Iranian Journal of Pharmaceutical Research: IJPR 18 (2019) 102.

[33] M.H. Shubbar, J.I. Penny. Effect of amyloid beta on ATP-binding cassette transporter expression and activity in porcine brain microvascular endothelial cells. Biochimica et Biophysica Acta (BBA)-General Subjects 1862 (2018) 2314-2322.

[34] W. Pan, J. Ryu, J. Shon, I. Song, K. Liu, Y. Sunwoo, W. Kang, J. Shin. Dietary salt does not influence the disposition of verapamil enantiomers in relation to efflux transporter ABCB 1 genetic polymorphism in healthy Korean subjects. Xenobiotica 38 (2008) 422-434.

[35] T.J. Powles, S. Ashley, A. Tidy, I.E. Smith, M. Dowsett. Twenty-year follow-up of the Royal Marsden randomized, double-blinded tamoxifen breast cancer prevention trial. Journal of the National Cancer Institute 99 (2007) 283-290.

[36] A. Paganini-Hill, L.J. Clark. Preliminary assessment of cognitive function in breast cancer patients treated with tamoxifen. Breast cancer research and treatment 64 (2000) 165-176.

[37] L. Chen, L. Zhu, M. Li, N. Li, F. Qi, N. Wang. Predicting the effects of different triazole antifungal agents on the pharmacokinetics of tamoxifen. AAPS PharmSciTech 20 (2019) 1-8.

[38] S.S. Sokar, T.A. El-Masry, M.E. El-Sayad, S.R. El-Afify. Pharmacokinetic and pharmacologic study of two P-glycoprotein modulating agents combined with doxorubicin. JPCS, 2012.

[39] H.-C. Seol, J.-S. Choi. Pharmacokinetic Interaction between Verapamil and Tamoxifen in Rats. YAKHAK HOEJI 49 (2005) 380-385.

[40] P. Limtrakul, S. Anuchapreeda, D. Buddhasukh. Modulation of human multidrug-resistance MDR-1 gene by natural curcuminoids. BMC cancer 4 (2004) 1-6.

[41] X. Li, J. Hu, B. Wang, L. Sheng, Z. Liu, S. Yang, Y. Li. Inhibitory effects of herbal constituents on P-glycoprotein in vitro and in vivo: Herb–drug interactions mediated via P-gp. Toxicology and applied pharmacology 275 (2014) 163-175.

[42] C.-H. Choi, G. Kang, Y.-D. Min. Reversal of P-glycoprotein-mediated multidrug resistance by protopanaxatriol ginsenosides from Korean red ginseng. Planta medica 69 (2003) 235-240.