RESEARCH ARTICLE

P Glycoprotein Mediated Drug Interaction between Digoxin and Orange Juice- Exploratory Study by *In-vitro* Approach

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ABSTRACT

P.gp is an efflux transporter that plays a vital role in drug transportation. Aim: This exploratory study is aimed to find the P-glycoprotein (P-gp) mediated drug interaction between digoxin and orange juice, conducted for a period of six months. The 3D structure of the human P-gp was predicted from the peptide by molecular threading using default settings. The structural analysis and verification server and the PDB were used to assess the stereo-chemical quality of these structures. The molecular structure of P-gp in a transport cycle has been investigated using a variety of methods. Molecular docking has been performed to predict the highest binding affinity to P gp with ligands of orange juice and ligands of ketoconazole. In 50% ethyl acetate extract of orange juice was prepared and a transcellular transport study was done using MDR1 transfectants and MRP2 transfectants LLC-PK1, LLC-GAS-COL150, LLC-pCI and LLC-MRP cells grown in M199 medium supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere of 5% CO2/95% air. The minimum inhibitory concentration is found to be 125 mcg/mL. Hence, the loading concentration along with orange juice is found to be 24.562 mcg/mL and the concentration less than that is found with poor sensitivity. Whereas the concentration above 125 mcg/mL loaded with orange juice is found to inhibit P-gp. The absorbance of orange juice and digoxin was measured at 470 nm. Since digoxin is not sensitive to orange juice beyond the concentration of 125 mcg/mL, it is considered as the MIC. Hence orange juice is a moderate p-gp Inhibitor and it escalates the serum digoxin toxicity. Orange juice is inhibiting the P-gp which increases the serum toxicity levels of digoxin.

Keywords: Digoxin, Orange juice, Colon cancer cells, P-Glycoprotein, *In-vitro* study, Toxicity, Interaction, Inhibitory effect.

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INTRODUCTION

P-glycoprotein was first identified as permeability (P)-glycoprotein because it made the cellularmembrane less permeable, which led to drug resistance, as discovered by Juliano and Ling in 1976.[1] Human P-glycoprotein has an outward-facing shape in its ATP-bound state, which belongs to the category of transport protein[2]. P-glycoprotein structures with an inward-facing conformation have been known for many years, but electron cryomicroscopy with a resolution of 3.4 A has recently been used to determine the outward-facing conformation of the human P- glycoprotein efflux ATP-driven conformational changes that remove xenobiotics and other medications from cells[3]. There are many different types of p-glycoprotein, including those found in humans, mice, *Cyanidioschyzon merolae*, and *C. elegans*. Human p-glycoprotein homology models with some of them having characteristics that were different from those of the mouse homology due to sequence variance.[4]

The efflux of medicines from tumor cells through ATP binding cassette (ABC) transporters, which function as integral membrane pumps, is the mechanism through which p-glycoprotein in cancer cells is activated 2 only one ATP is hydrolyzed at a time despite the presence of two active ATPase sites.[5] The transport cycle of the substrates (drugs) that are bound to the transmembrane binding pocket of p-glycoprotein in an inward-facing conformation are converted into an outward-facing conformation that can release the substrates to the extracellular environment, which happens as a result of the hydrolysis of ATP and made possible by the dimerization of NBD's that initially led to the outward facing conformation, and it then returns to the initial inward facing state and closes the cycle.[6] Multi-drug resistance occurs when cancer cells become resistant to a variety of chemicals. Not only in cancer but in other conditions as well, multidrug resistance results in therapeutic failure.[7] Over the past few decades, MDR inhibitors from almost three generations have been tested in clinical settings. In 1973, Keld Dano demonstrated how energy depended on the decreased levels of drug accumulation in tumor cells.[8]

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Together with grapefruit juice, orange juice is considered one of the p-glycoprotein inhibitors that belong to the third generation of p-glycoprotein inhibitors. The ingredients in orange juice include sucrose, naringin, narirutin, malic acid, hydroxycinnamic acid, glucose, fructose, ferulic acid, didymin, citric acid, ascorbic acid, and hesperidin, among others. Orange juice inhibits p- p-glycoprotein for a very long time. Another p-glycoprotein inhibitor, such as grapefruit juice and its constituents 6', 7' dihydroxybergamotten (DHBG), bergamot tan (BG), and other furanocoumarin derivatives, effectively suppresses p-glycoprotein activity. Tangerine (TAN), 3, 3, 4, 5, 6, 7, and 8-heptamethoxylavone (HM), and nobiletin (NBL), which are found in orange juice, block p-glycoprotein.[9] Ketoconazole, an imidazole derivative classified as an antifungal, is apotent p-glycoprotein inhibitor.[10] The kidney epithelial cells exhibit the effects of orange juice on drug transport using the MDR1 protein, also referred to as efflux transporters.[11]

The p-glycoprotein substrates are:

Pharmacological classification – Drugs

Antacids - Cimetidine, Ranitidine

Antiemetic - Ondansetron

Anti-tumor - Paclitaxel, Vincristine, Doxorubicin, Vinblastine, Imatinib. Beta-Adrenoreceptor antagonist – Reserpine Immunosuppressants - Cyclosporin, Sirolimus Cardiac drugs/ Anti-arrhythmic - Digoxin

Digoxin has a molecular weight of 780.9 and a log *p-value* of approximately 1.26.[11] It is soluble inalcohol or diluted alcohol and sparingly soluble in water. Digoxin pharmacokinetics may be affected because p-glycoprotein, which is found on intestinal cell surfaces, is an efflux transporter substrate.[12] By enhancing its efflux in the intestine, p-glycoprotein can be stimulated by other medications to lessen the effects of digoxin.

MATERIALS AND METHODS

The study was conducted as a prospective exploratory study at JSS College of Pharmacy Udhagamandalam, for a period of 3 months. To study the interaction between orange juice and digoxin, *in-vitro* study was carried out using $CaCO^{-2}$.

Extraction of various components from orange juice was performed. Orange juice was mixed with 600 mL of ethyl acetate and shaken vigorously for 10 minutes. After the removal of an aqueous phase, the organic layers were evaporated to dryness. The residue was dissolved in 10 mL of methanol and stored at 20%. For experiments, the methanol was evaporated under a nitrogen stream and the residue was dissolved in dimethyl sulfoxide (DMSO) and diluted with buffer to give a final concentration of 50% of juice. The DMSO conc. was 0.5%. $CaCO^{-2}$ cells (Intestinal epithelial cell line) were obtained $CaCO^{-2}$ cells were grown in M199 medium supplemented with 10% fetal calf serum at 37% C in a humidified atmosphere of 5% CO_2/95% air. $CaCO^{-2}$ cell line was obtained by selection with 150 ng mL^{-1} colchicine and cultured in M199 medium supplement with 10% fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2 95% air. $CaCO^{-2}$ (Intestinal epithelial cell line) were obtained by selection with 800 µg mL^{-1} G418 and cultured in M199 medium supplemented with 0% fetal calf serum at 37% in a humidified atmosphere 5% CO_2/95% air. In cells, $CaCO^{-2}$, p-glycoprotein and MRP2 are located at the apical membrane. Transcellular transport study MDR1 transfectants and MRP2 transfectants $CaCO^{-2}$ cells were seeded on a polycarbonate membrane. These cells were grown for three days and the culture medium was replaced with the fresh one. Then the culture medium was removed and cells were washed once or twice with transport buffer and measurement of the transport was done.

Uptake experiments were performed in 250 µL of incubation buffer containing 20 nm saquinavir in the presence or absence of an inhibitor, and ethyl acetate extract of orange juice is taken. After incubation, the cells were washed three times with ice-cold buffer to stop the uptake. Then the cells are dissolved with NaOH and neutralized with 6M HCl and measurement of transport was performed, and then calculations are done.

RESULTS

Table 1 and Figure 1 suggests an inverse relationship between the concentration of orange extract and cytotoxicity, meaning that as the concentration decreases, cytotoxicity tends to decrease as well. Here's a general interpretation: As the concentration of orange extract decreases, the cytotoxicity decreases, indicating a potential dose-dependent effect. At higher concentrations (1000 and 500), the cytotoxicity is

Table 1: The concentration of the orange juice extract taken and their respective cytotoxicity

Orange extract	
Concentration	*Cytotoxicity*
1000	45.23
500	38.632
250	29.656
125	24.562
62.5	20.689
31.25	16.756
15.625	12.653
7.8	9.234

Table 2: The concentration of digoxin taken and their respective cytotoxicity

Digoxin	
Concentration	*Cytotoxicity*
1000	92.24
500	59.518
250	41.74
125	26.185
62.5	18.489
31.25	15.73
15.625	7.954
7.8	6.925

Table 3: The loaded concentration of digoxin and orange juice and their percentage difference

Loaded concentration	*Cell uptake*	*Digoxin+orange juice concentration*	*Difference*	*%difference*
1000	92.24	45.23	47.01	-50.96
500	59.518	38.632	20.886	-35.091
250	41.74	29.656	12.084	-28.950
125	26.185	24.562	1.623	-6.198
62.5	18.489	20.689	2.200	+11.898
31.25	15.73	16.756	1.026	+6.522
15.625	7.954	12.653	4.699	+59.077
7.8	6.925	9.234	2.309	+33.342

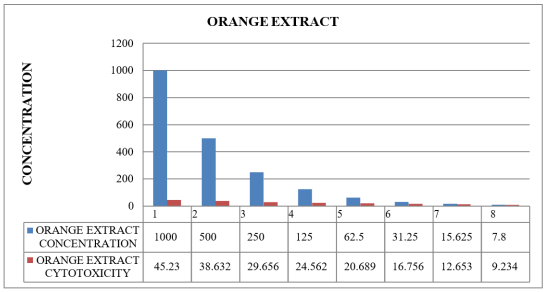

Figure 1: The graphical representation of the orange juice extract concentration taken and their respective cytotoxicity

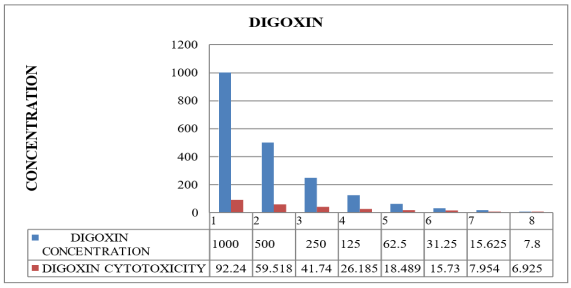

Figure 2: The graphical presentation of the digoxin extract concentration taken and their respective cytotoxicity

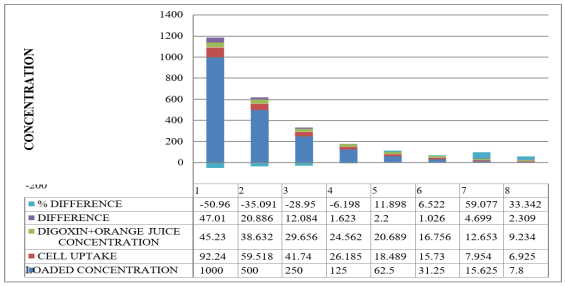

Figure 3: The graphical representation of the loaded concentration of digoxin and orange juice and their percentage difference

relatively higher (45.23 and 38.632, respectively), while at lower concentrations (31.25 and below), the cytotoxicity is considerably lower (16.756 and 9.234, respectively). As the concentration of digoxin decreases, the cytotoxicity also decreases, suggesting a potential dose-dependent effect. At higher concentrations (1000 and 500), the cytotoxicity is relatively higher (92.24 and 59.518, respectively), while at lower concentrations (31.25 and below), the cytotoxicity is considerably lower (15.73 and 6.925, respectively). This pattern indicates that there may be a concentration-dependent response to digoxin, and further investigation may be required to understand the specific relationship between digoxin concentration and cytotoxicity. This is sown in Table 2 and Figure 2. The Table 3 and Figure 3 provides insights into the potential interactions between digoxin and orange juice at different concentrations, revealing varying effects on cell uptake.

DISCUSSION

In this *in-vitro* study, we embarked on a journey to unveil valuable insights into the potential interaction between the cardiac medication, digoxin and a seemingly innocuous beverage, orangejuice, with a focus on the key player in the dynamic interaction of p-glycoprotein which is a transporter protein, known for its involvement in drug interactions. Our quest began with the determination of the minimum inhibitory concentration (MIC) for digoxin, a critical baseline parameter, which we found to be 125 mcg/mL. Armed with this knowledge; we entered into the world of drug synergy with orange juice. As we mixed digoxin and orange juice, an intriguing story unfolded. The loaded concentration, an amalgamation of the 2 entities, was revealed to be 24.562 mcg/mL. This concentration lies within the fascinating realm between the MIC and 4 times the MIC for digoxin, i.e., 125 mcg/mL. At concentrations below 24.562 mcg/mL, our experimental concoction appeared to have a limited impact on p-glycoprotein to continue its role in the transport of digoxin. However, concentrations exceeding 125 mcg/mL of the loaded concentration, when mixed with orange juicewere found to inhibit p-glycoprotein. This outcome ignites a spark of curiosity and raises significant questions. * How does the co-administration of orange juice affect the pharmacokinetics of digoxin at a concentration exceeding the MIC? What are the clinical implications of this p-glycoprotein-mediated interaction? Suggests that the interaction between digoxin and orange juice may affect the bioavailability and pharmacokinetics of digoxin, particularly at higher concentrations. Further investigations are warranted to elucidate the precisemechanisms underlying this interaction and its potential our research underscores the complexity of drug interactions and the potential for seemingly innocuous substances like orange juice to influence the behavior of medications. While this *in-vitro* study offers a glimpse into the interplay of digoxin, orange juice, and, it also emphasizes the need for further exploration. In conclusion, our exploration into the p-glycoprotein-mediated drug interaction between digoxin and orange juice has unveiled a captivating narrative. This interaction, when delved into deeply, could have significant ramifications for the field of pharmacology and clinical medicine. Our journey has just begun, and the full implications of this interaction may only be fully appreciated

in future research and clinical practice.

CONCLUSION

Our *in-vitro* study on the interaction between digoxin and orange juice focusing on p-glycoprotein mediated drug interactions has yielded significant findings. The MIC for digoxin was determined to be 125 mcg/mL. When digoxin was co-administered with orange juice, we observed a loaded concentration of 24.562 mcg/mL. This loaded concentration lies between the MIC and four times the MIC which suggests a noteworthy phenomenon. The concentration below 24.562 mcg/mL did not significantly inhibit p-glycoprotein, indicating that p-glycoprotein remained active in transporting digoxin. However, the concentrations above 125 mcg/mL of the loaded concentration, along with orange juice, demonstrated inhibitory effects on p-glycoprotein. Orange juice is inhibiting the P-gp which increases the serum toxicity levels of digoxin.

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