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INFLUENCE OF NATURAL BIOENHANCER QUERCETIN ON PERMEABILITY CHARACTERISTICS OF GRANISETRON ACROSS GOAT INTESTINAL MEMBRANE MODEL

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ABSTRACT

The purpose of the present study was to explore the effect of presence of bioenhancer quercetin on membrane permeability of poorly permeable Granisetron HCl, across goat intestinalmembrane using Franz diffusion cell. The effect of quercetin was investigated upon co- administration of different concentrations (2, 6, 10, 14 and 18mg) of quercetin and upon pre- treatment at different time periods (30, 60 and 120 min) on goat intestinal permeability of Granisetron HCl in phosphate buffer pH 7.4. Data obtained from permeability studies were used to calculate percentage cumulative drug release (% CDR), apparent permeability (Papp), flux (J) and enhancement ratio (ER). The pretreatment studies with quercetin for 1 and 2 hoursculminated in a notable enhancement in the permeability of Granisetron HCl. The % CDR values after 60 min (55.70%) and 120 min (49.13%) of pre-treatment are higher than control drug (41.78%). This implies that a 60-minute pre-treatment shows a more optimal effect. The study revealed that co-administration of the different concentrations of quercetin has detrimental effect on the membrane permeability of drug. However, upon pre-treatment at different periods, it improves the permeability of Granisetron HCl.

KEYWORDS: Poorly permeable drug, Granisetron, Bioenhancer, Quercetin, Franz diffusion study, *Ex vivo* permeability study.

INTRODUCTION

Oral delivery of BCS Class III and IV drugs is often hindered by low intestinal permeability, resulting in reduced drug absorption and poor bioavailability. This challenge is due to unfavourable physicochemical properties of the drugs, such as large molecular weight, low octanol/water partition coefficient, high hydrophilicity, and high log P values, along with the presence of multiple hydrogen bonding groups and a large polar surface area. Additionally, secretory transporters like P-glycoprotein (P-gp) actively efflux drug molecules back into the intestinal lumen, further limiting their absorption. These factors make it difficult to improve drug permeability using conventional methods.[1,2]

To overcome these challenges, natural herbal bioenhancers have emerged as a promising solution. Bioenhancers can increase drug permeability, enhancing bioavailability and bio efficacy at lower doses. This approach not only reduces the required drug dosage but also decreases dosing frequency, treatment costs, and potential side effects, thereby improving patient compliance. Among these bioenhancers, Quercetin has been reported to act as a modulator of P-gp, inhibiting the gastro-intestinal P-gp efflux pump and the metabolizing enzyme CYP3A4 in vitro offering a safe and innovative strategy for enhancing oral drug delivery in the pharmaceutical and healthcare industries.[3-6]

Granisetron is a selective 5-HT_3 receptor antagonist used to prevent nausea and vomiting caused by chemotherapy and radiotherapy. It works by reducing vagus nerve activity, which triggers the vomiting center. It has moderate tissue distribution and binds to plasma proteins at 65-75%. The absolute bioavailability of the oral formulation is approximately 60-65% due to first-pass metabolism.[7] Granisetron is mainly metabolized by the CYP1A1 and CYP3A enzymes and is likely a substrate of the ABCB1 transporter P-gp[8,9] which makes it an idealcandidate for administration with bioenhancers.

Bioenhancers are substances that increase the bioavailability and effectiveness of active compounds without having any pharmacological action at their own dose. They can improve the absorption of drugs, vitamins, nutrients, and toxins, depending on their mechanism of action. Synthetic bioenhancers are typically developed using medicinal chemistry techniques to enhance their potency and selectivity. Examples include cyclodextrins, polyethylene glycol (PEG) derivatives, and nanoparticle-based delivery systems. However, recent studies onepithelial permeability have shown that many bioenhancers exhibit more toxicity than efficacy, leading to a decline in their use. There is now a growing need to develop safer and more effective natural bioenhancers for therapeutic applications. Examples of natural bioenhancers include curcumin, quercetin, piperine, genistein, and aloe vera. These compounds offer severaladvantages, such as lowering drug dosage, reducing the risk of drug resistance, and minimizingadverse side effects or toxicity. This is particularly beneficial for medications like anti-cancer drugs, where enhanced bioavailability can significantly improve drug efficacy.[10,11]

Bioenhancers work through several mechanisms: they can inhibit metabolic enzymes like cytochrome P450, reducing drug metabolism and clearance, which increases drug bioavailability. They also enhance drug solubility by forming complexes with drug molecules or altering their properties, improving absorption. By modulating drug transporters, such as inhibiting P-glycoprotein, bioenhancers prevent drug extrusion from cells, raising their concentration. They may protect drugs from degradation via antioxidant properties and stimulate blood circulation, boosting delivery to target tissues. Additionally, bioenhancers canincrease gut permeability, allowing drugs to enter the bloodstream more easily.[12]

Quercetin, a natural bioenhancer found in fruits and vegetables, boosts drug bioavailability andeffectiveness. It works by inhibiting cytochrome P450 enzymes and modulating drug transporters like P-glycoprotein, reducing drug breakdown and improving intestinalpermeability. This enhances drug concentration in the bloodstream and improves drug delivery and efficacy.[12-16]

It is hypothesized that co-administration or pre-treatment with a bioenhancer quercetin could improve granisetron's permeability, bioavailability, plasma concentration, and biological effects. No experiments have been reported on optimizing the ex vivo permeability characteristics of granisetron in the presence of quercetin across goat intestinal membrane. The beneficial effects of the quercetin sparked our interest in exploring its impact on goat intestinal permeability of granisetron using a Franz diffusion cell in this study.

MATERIALS AND METHODS

Materials

All experimental procedures used chemicals of analytical grade. Granisetron was generously provided by Panchsheel Organics Limited in Mumbai, India, while Quercetin was supplied by High Media Laboratories Pvt. Ltd. in India. Deionized double-distilled water from Symbiosis Pvt. Ltd. was used in the study. Fresh goat intestine, sourced from the local slaughterhouse, was utilized within one hour of the goat's slaughter.[17]

Method

Optimization of ex-vivo permeability characteristics of Granisetron HCl

I. Preparation of receptor fluid

Phosphate buffer (pH 7.4) was prepared as per Indian Pharmacopoeia Monograph and used asa receptor fluid.

II. Preparation of goat intestine

Ex-vivo permeability studies were conducted using freshly excised goat intestine in phosphatebuffer (pH 7.4), as the goat jejunum is a reliable model for predicting oral absorption in humans (Garg et al. 2011). The freshly cut intestinal membrane was washed with phosphate buffer andsectioned into 3 cm² pieces. The tissue was kept viable for up to 1 hour in the buffer with oxygen supplied by an aerator. During pretreatment, the goat intestine was exposed to three different concentrations of bioenhancer for 30, 60, and 120 minutes.

III. Preparation of control: Granisetron HCl (10mg) was used as a control for this study (Sample code G).

IV. Preparation of test sample

A fixed dose of 10 mg Granisetron HCl was used as the test sample, while co-administration studies were conducted with varying concentrations of the natural bioenhancer Quercetin, as detailed in Table 1.

V. *Ex- vivo* permeability study

Ex- vivo permeability study utilized freshly dissected goat intestinal tissue, because goat jejunum is a good predictor of human oral absorption. The tissue was sliced into 3.2 cm2 sections with a thickness of 500-600 μm and supplying with oxygen via an aerator and pH 7.4 phosphate buffer to keep it alive. Granisetron at a dosage of 10 mg served as the control sample. In the experimental sample, a consistent 10 mg dose of granisetron was used, and five different concentrations of quercetin were co-administered alongside granisetron, as outlined in Table 1.

The study involved studying the permeability of pure Granisetron in Franz diffusion cells usingexcised goat intestine with and without the bioenhancer quercetin. The goat intestine's mucosal side was placed facing upward between the chambers of the diffusion cell, which had a capacity of 10ml in the receptor chamber and an available diffusion area of 3.14 cm^2. For the pretreatment, the intestine was exposed to the quercetin solution for 30, 60, and 120 minutes. Subsequently, a mixture of granisetron and quercetin was introduced into the donor compartment. The goat intestine was cut into 3cm sections and positioned between the donorand receiver compartments with the mucosal side facing upward, secured with springs. The 10ml capacity receiver compartment was filled with saline phosphate buffer of 7.4pH. The receptor fluid, maintained at a temperature of 37°C ± 10°C, was constantly stirred at 100 rpm with a magnetic stirrer bead for 6 hours. Every half hour, 1ml of solution from the receiver chamber was withdrawn and diluted with the buffer solution. These samples were tested for UV absorbance using a UV spectrophotometer at 302nm.[12,13]

Table No. 1: Composition of test sample: Granisetron HCl + Quercetin.

Sample Code	Granisetron HCl (mg)	Quercetin (mg)
G	10	…
CoQ1	10	2
CoQ2	10	6
CoQ3	10	10
CoQ4	10	14
CoQ5	10	18

The data collected from the permeability study for each test and control sample were utilized to calculate permeability parameters such as % cumulative drug release (%CDR), expressed as mean ± SD, apparent permeability (Papp), Flux (J), and enhancement ratio (ER) (n=3) usingstandard formulas. Permeability coefficient (apparent permeability):[18-19]

$$papp\left(\frac{cm}{s}\right) = \left(\frac{VA}{[area \times time]}\right) \times \left(\frac{[Drug])acceptor}{[Drug]donor}\right)$$

Where,

VA = volume in acceptor chamber,

Area = intestinal membrane surface area,

Time = total transport time.

$$Flux(J)\left(\frac{\frac{mg}{cm2}}{hr}\right) = \frac{mass\ diffusing}{surface\ area \times time}$$

Enhancement Ratio (ER) = Papp of combination / Papp of control

RESULTS AND DISCUSSION

The ex vivo permeability study of Granisetron included testing its permeability in the presence(co-administration / pre-treatment) and absence (control G) of quercetin. Calibration curve ofGranisetron in phosphate buffer pH 7.4 was shown in figure no. 1 and the data was shown in table no. 2. During the co-administration process, it was observed that the permeability of granisetron did not improve at all the concentrations of bioenhancers. (% CDR 28.12) at 6 mg of quercetin; CoQ2 batch). The minimum value observed (% CDR 7.43 %) was at 14 mg of quercetin (CoQ4 batch), compared to the control sample Granisetron (G) (% CDR 47.57 at 10mg). Since quercetin acts as an inhibitor of P-gp, co-administration of quercetin negatively influences the permeability characteristics of Granisetron hydrochloride.

Results of the permeability study of test and control sample are shown in table no. 3. The calculated values of permeability parameters % CDR, Papp, J, and ER up to 6 hours for all samples were also decreased significantly as shown in table no. 4. The impact of different concentrations of quercetin on the % CDR of Granisetron HCl is presented in Figure no.2.

Table No. 2: Data for Calibration curve of Granisetron in phosphate buffer pH 7.4.

Sr. No.	Concentration (µg/ml)	Absorbance at 302nm
1	2	0.098
2	4	0.164
3	6	0.239
4	8	0.332
5	10	0.408

Absorption maxima	302 nm
Slope (m)	0.0394
Intercept (c)	0.0118
Coefficient of correlation (r^2)	0.9968

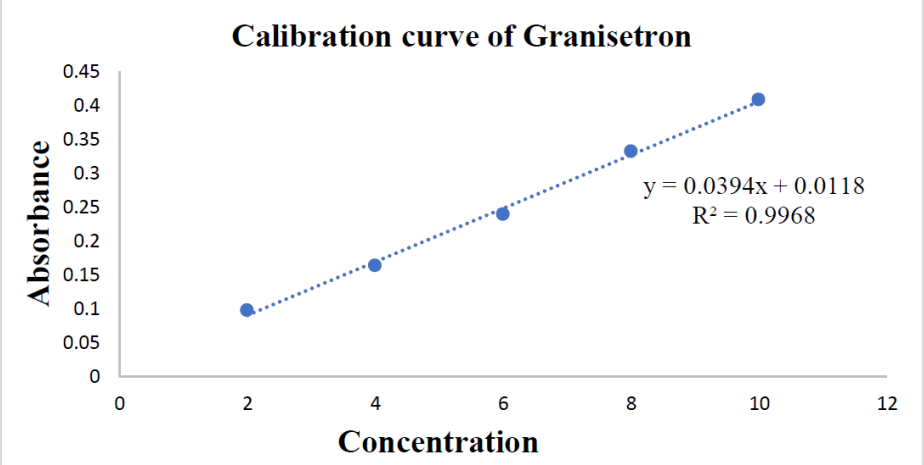

Figure No. 1: Calibration curve of Granisetron in phosphate buffer pH 7.4

Table No. 3: % CDR of Granisetron hydrochloride from control and coadministration study.

Time (hr)	G (%)	CoQ1 (%)	CoQ2 (%)	CoQ3 (%)	CoQ4 (%)	CoQ5 (%)
0	0	0	0	0	0	0
0.5	8.279187817	2.263959	8.431472	1.350254	1.172589	0.411168
1	10.11269036	2.845685	10.59442	3.109645	3.904061	0.629949
1.5	15.53502538	3.691371	15.60508	4.473096	3.52132	1.983249
2	19.41928934	5.229949	17.36447	6.273096	5.042132	2.349239
2.5	25.47106599	6.281218	20.27868	7.298985	6.659391	2.809137
3	31.12182741	7.520305	22.86041	8.576142	3.903553	3.497462
3.5	36.91979695	8.807614	25.64518	10.53096	5.005584	4.536041
4	37.14416244	9.506091	26.48579	12.46294	3.152284	8.594924
4.5	38.8	10.26041	26.8264	13.46853	12.19695	9.445685
5	41.82639594	11.05025	27.56548	14.53503	6.253299	9.877665
5.5	44.74111675	11.82741	27.48731	15.54061	6.573604	10.22843
6	47.57461929	12.6401	28.11726	16.55381	7.43198	10.69594

G: Granisetron hydrochloride (Control); CoQ1: Co-administration with 2mg of quercetin; CoQ2: Co-administration with 6 mg quercetin; CoQ3: Co-administration with 10 mg quercetin; CoQ4: Co-administration with 14 mg quercetin; CoQ5: Co-administration with 18 mg quercetin.

Table No. 4: Permeability parameters of Granisetron hydrochloride from control and coadministration study.

Samples	%CDR	Papp $\times 10^{-7}$cm/s	J (mg/cm^2/hr)	ER
G	47.57	1.92	10.09	--------
CoQ1	12.64	2.68	2.68	0.16567
CoQ2	28.11	0.83	5.9666	0.43103
CoQ3	16.55	0.42	3.5128	0.21860
COQ4	7.43	0.17	1.5771	0.08847
CoQ5	10.69	0.25	2.2697	0.13198

G: Granisetron hydrochloride (Control); CoQ1: Co-administration with 2mg of quercetin; CoQ2: Co-administration with 6 mg quercetin; CoQ3: Co-administration with 10 mg quercetin; CoQ4: Co-administration with 14 mg quercetin; CoQ5: Co-administration with 18 mg quercetin.

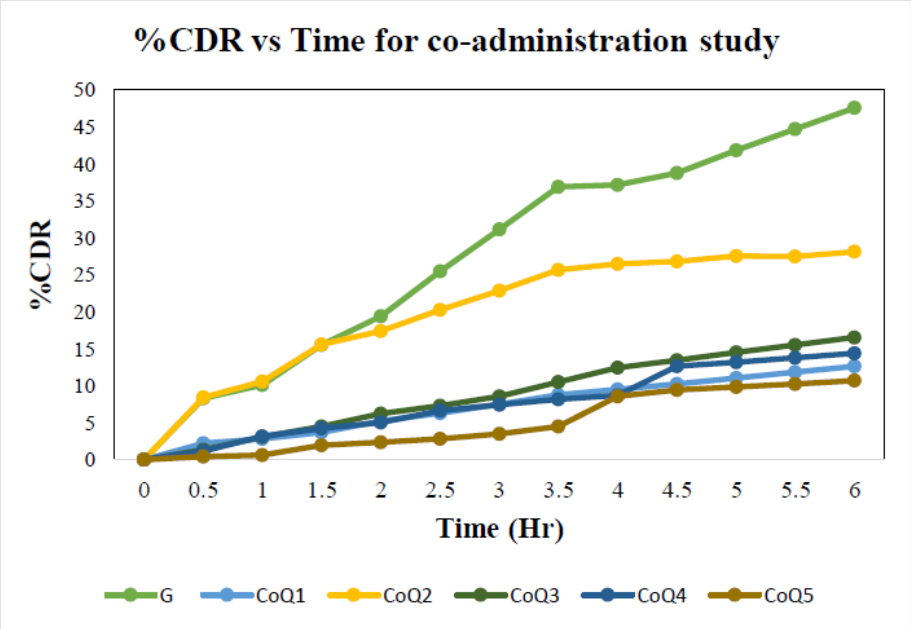

Figure no. 2: % CDR of Granisetron hydrochloride from control and co-administration study.

Influence of pre-treatment of quercetin on permeability profile of Granisetron HCl

The effect of different pre-treatment time of quercetin on % CDR of Granisetron is presented in the table no. 5. The Figure no. 3 illustrates the impact of various pre-treatment durations (30, 60, and 120 minutes) of quercetin on the % CDR of Granisetron for all samples. Pre-treating with quercetin for 1 and 2 hours has demonstrated positive effects by enhancing the permeability of Granisetron. The % CDR values after 1 hour (55.7015) and 2 hours (49.13) of pre-treatment are higher than the % CDR of the control drug (41.7898). This implies that a 60-minute pre-treatment shows a more optimal effect compared to the 120-minute pre-treatment.

The results of the *ex-vivo* permeability studies of Granisetron hydrochloride in the presence (pre-treatment) and absence (control) of quercetin were shown in Table 11.

Table no. 5: Permeability parameters of Granisetron hydrochloride from control and pre-treatment studies.

Time hr	G	PreQ1	PreQ2	PreQ3
0	0	0	0	0
0.5	8.27918782	6.121827411	5.1573604	4.979695431
1	10.1126904	7.650761421	8.3969543	8.031472081
1.5	15.5350254	9.494416244	15.758376	10.40304569
2	19.4192893	11.77969543	15.871066	13.57664975
2.5	25.471066	14.44568528	24.232487	16.17664975
3	31.1218274	16.49238579	31.629442	20.28426396
3.5	36.919797	19.09746193	36.630457	23.91472081
4	37.1441624	21.85482234	41.210152	27.7786802
4.5	38.8	24.33299492	43.398985	35.22639594
5	40.1705584	25.63350254	47.20203	41.2680203
5.5	41.3939086	27.41116751	51.964467	45.16243655
6	41.7898477	29.23959391	55.701523	49.13299492

Data represents: G= Plane granisetron HCl, PreQ1= Pre-treatment with Quercetin for 30min,PreQ2= Pre-treatment with Quercetin for 60min, PreQ3= Pre-treatment with Quercetin for 120min.

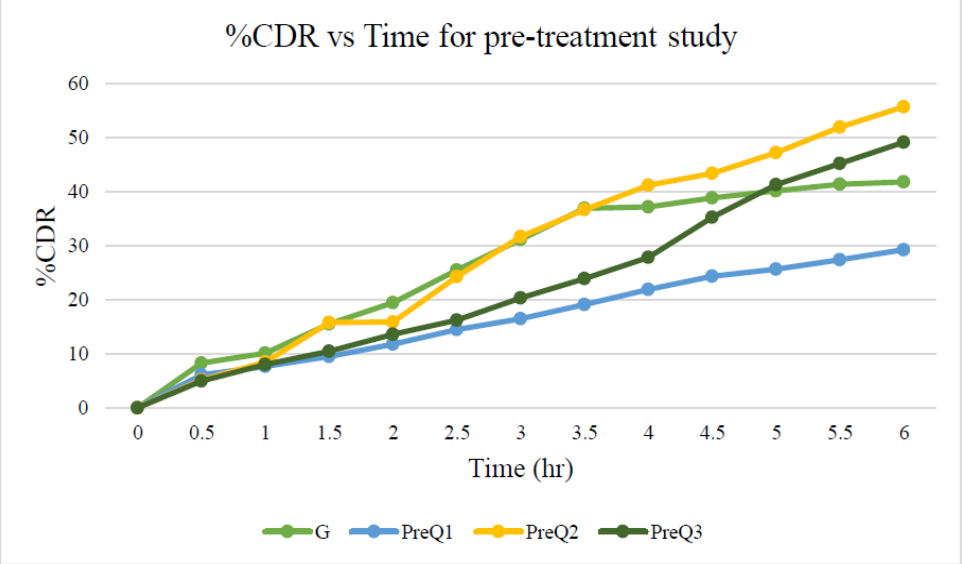

Fig. 3: Effect of pre-treatment of quercetin for 30 min, 60min, 120min on % CDR.

As granisetron is a substrate of the ABCB1 transporter P-gp and also it undergoes pre-systemicmetabolism due to CYP 3A4 and CYP 1A1 enzymes, it has shown poor permeability characteristics (47.57 % CDR). P-gp might be involved in the efflux of granisetron back againinto intestinal lumen, leading to poor absorption and thus low bioavailability (7-9).

It has been reported that quercetin acts as a modulator of P-gp and can inhibit the gastrointestinal P-gp efflux pump as well as the metabolizing enzyme CYP3A4 in vitro. In a study investigating the enhancement of diltiazem's oral bioavailability in rabbits, pre-treatment and co-administration with quercetin were examined. The findings showed a significant increase in diltiazem bioavailability when quercetin was given as a pre-treatment compared to the control group, whereas no notable improvement was observed with co-administration. This lack of effect during co-administration could be due to the formation of a complex in the gastrointestinal lumen resulting from the interaction between quercetin and diltiazem at a high dose of quercetin (20 mg). The bioavailability enhancement observed with pre-treatment, where quercetin was administered 30 minutes prior to diltiazem, is likely due to its early gastrointestinal absorption, allowing inhibition of CYP3A4 and the P-gp efflux pump before diltiazem administration. However, this

effect was not seen with co-administration (12, 20-23).Similarly, in our ex vivo permeability study on goat intestine, an increase in the permeability of granisetron was observed following pretreatment with quercetin.

CONCLUSION

In the present research, the beneficial effect of quercetin pre-treatment may enhance the permeability of granisetron, leading to improved therapeutic efficacy. The limitation of granisetron's poor intestinal permeability could be effectively overcome by pre-treating with the bioenhancer quercetin, with a 60-minute pre-treatment period proving optimal for increasing the permeability of the poorly bioavailable granisetron, reaching up to 55.70% CDR.This effect is likely due to quercetin's inhibition of metabolizing enzymes and the P-gp efflux pump, which act as an absorption barrier for granisetron.

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CONFLICT OF INTEREST

The author(s) declare(s) that they have no declaration of interests to disclose.

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