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Research Article

In Vitro Herb–Drug Pharmacokinetic Interaction Study of Cisplatin with Holy Basil (*Ocimum sanctum*)

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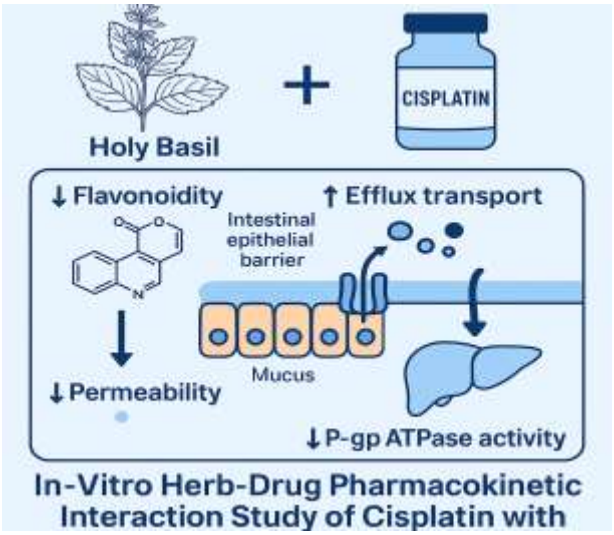
Abstract

Herbal supplements are widely used alongside conventional chemotherapy, raising concerns about herb–drug interactions that may alter pharmacokinetics. Holy Basil (*Ocimum sanctum*, Tulsi) contains bioactive phytochemicals capable of modulating enzymes and drug transporters. This study evaluates the in-vitro pharmacokinetic interactions between Holy Basil extract and cisplatin using Caco-2 permeability assays, P-glycoprotein ATPase activity assays, and physicochemical characterization. Holy Basil significantly reduced the absorptive permeability (Papp AP→BL ↓37.5%) and increased efflux ratio (↑125.7%), indicating enhanced transporter-mediated efflux. ATPase assays revealed concentration-dependent inhibition of P-gp activity, confirming transporter involvement. Microsomal stability suggested increased metabolic turnover of cisplatin. Overall, Holy Basil significantly alters cisplatin permeability and efflux, suggesting a potential reduction in its bioavailability if co-administered clinically.

Keywords: Cisplatin, Holy Basil, Herb–drug interaction, P-glycoprotein, Caco-2 permeability, Pharmacokinetics



1. Introduction

Herbal medications are extensively utilized in conjunction with contemporary chemotherapy treatments, especially in Asian nations, where traditional herbal therapies are profoundly embedded in cultural health practices. From the past many years, cancer patients use many herbal extracts and their products with cytotoxic medicines to overcome or prevent the adverse effects or side effects and to boost their immune system [1,2]. This co administration increases the tendency of important clinical concerns about herb drug interactions which alter drug's pharmacokinetic processes. These interactions change the absorption, distribution, metabolism and elimination kinetics of the drug by changing the transporter functions, functioning of metabolizing enzymes, and detoxification mechanisms [3] From the ancient times in Ayurveda Holy basil (Ocimum Sanctum linn.) is most widely used medicinal plant for its anti-inflammatory, antidiabetic, antibacterial and anticancer characteristics. Phytochemical analysis of Holy basil confirms presence of terpenoids, flavonoids, phenolic component, essential oils like eugenol, rosmarinic acid, ursolic acid, apigenin and many which exhibits significant bioactivity [5,6]. The phytoconstituents regulates various metabolizing enzymes like cytochrome P450 isoenzymes, ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp) and MRP 2 enzymes [7,8]. The bioactives of Holy basil can affect the pharmacokinetics of co-administered anticancer drugs. Cisplatin is the drug of choice for many solid tumors of ovarian, lung, colon, teseticular, cervical and bladder. Cisplatin on administration binds to DNA causing apoptosis. Cisplatin also gets affected by various membrane transport proteins [10,11]. These transporters effects distribution of drugs and decreases therapeutic efficacy and is also cause of intracellular cisplatin accumulation. Several plant-derived antioxidants, including those found in Holy Basil, can chelate platinum ions, scavenge reactive oxygen species, modulate glutathione levels, or affect transporter expression, potentially altering the cellular processing and pharmacokinetics of cisplatin [12,13]. Considering these mechanistic factors, assessing the possible interaction between Holy Basil and cisplatin is of considerable pharmacological relevance. In vitro methods, including Caco-2 monolayers, liver microsomal stability assays, plasma protein binding investigations, and P-gp ATPase functional assays, offer controlled and mechanistic insights into phases of drug disposal potentially influenced by herbal constituents [14,15]. These models eradicate complicated systemic variables and provide focused evaluation of intestinal permeability, efflux transporter function, drug

metabolism, and binding affinity. Despite the prevalent utilization of Tulsi supplements among cancer patients, there exists a paucity of systematic assessment regarding its influence on cisplatin disposal at both cellular and molecular levels. This study aimed to examine the in-vitro pharmacokinetic interaction between Holy Basil extract and cisplatin utilizing several mechanistic models. The objective is to clarify whether Holy Basil influences the absorption, efflux, metabolic stability, or protein-binding properties of cisplatin, therefore forecasting possible clinical ramifications during concurrent administration.

2. Materials and Methods

2.1 Chemicals and reagents

Cisplatin (≥99% purity) was acquired as an analytical standard. Holy Basil extract (standardized ethanolic extract) was obtained from a reputable herbal supplier. Caco-2 cells were acquired from NCCS Pune. HPLC-grade methanol, acetonitrile, formic acid, and water were procured from Merck India. All compounds were of analytical quality.

2.2 Procurement of selected standardized herbal extract

The dried leaves of Ocimum sanctum (Holy Basil) underwent botanical verification using macroscopic and microscopic analysis to confirm accurate plant identification and eliminate the possibility of adulteration or substitution. Subsequent to authentication, the leaves were meticulously cleansed to eliminate dust and extraneous particle matter, then air-dried in the shade at a regulated room temperature to safeguard thermolabile phytoconstituents. The completely desiccated leaves were coarsely pulverized with a mechanical grinder to augment the surface area for solvent infiltration and improve extraction efficacy. A Soxhlet extraction was conducted utilizing 95% ethanol as the solvent, due to its capacity to solubilize a wide array of polar and semi-polar phytochemicals, such as polyphenols, flavonoids, terpenoids, and alkaloids. Approximately 100 g of powdered plant material was placed in a cellulose cap and positioned in the Soxhlet apparatus, while ethanol was heated to reflux in the solvent reservoir. Continuous hot percolation was conducted for 6 to 8 hours until the siphon mechanism signaled the presence of clear solvent, indicating complete extraction of the bioactive compounds. The resultant ethanolic extract was filtered using Whatman No. 1 filter paper to eliminate insoluble plant residues. The filtrate was subsequently concentrated under reduced pressure with a rotary evaporator at 40–45°C

to avert thermal destruction of heat-sensitive chemicals. The semi-solid extract was subsequently dried in a vacuum desiccator to eliminate residual solvent, resulting in a viscous, dark-green mass typical of Holy Basil extract. The % yield was determined using the initial dry weight of the plant material.

2.3 Phytochemical Characteristics of Sample

The qualitative phytochemical analysis of Holy Basil extract identified numerous principal types of secondary metabolites, such as flavonoids, polyphenols, triterpenoids, tannins, alkaloids, and volatile essential oils. The phytochemical complexity of Holy Basil highlights its capacity to engage with several pharmacokinetic processes. The existence of these bioactive elements necessitates a comprehensive assessment of their impact on cisplatin permeability, efflux regulation, and metabolic stability in in vitro pharmacokinetic models.

2.4 Physicochemical Characteristics of Sample

Initial physicochemical assessments—comprising extractive values (both water-soluble and alcohol-soluble), moisture content, and pH—were conducted in accordance with recognized pharmacognostic and pharmacopeial protocols [13,14]. These tests yield critical data concerning the quality, purity, and solubility properties of the extract. The moisture content was measured to evaluate extract stability and vulnerability to microbial destruction. The pH was assessed to evaluate potential compatibility with biological systems and excipients in medicine formulation.

2.5 Caco-2 permeability study

The Caco-2 permeability assay was performed utilizing fully differentiated Caco-2 monolayers, grown for 21 days to ensure the complete development of tight junctions and polarization of the epithelial layer. The culture media was substituted every 48 hours throughout the experiment. The cells were divided into two experiments a control group which received cisplatin alone (10µg/mL) and test group which received cisplatin (10µg/mL) and co-administration of holy basil (200µg/mL).The solution to administer was administered to the apical compartment to stimulate lumen exposure while the basolateral chamber contained fresh transport buffer. Samples from both compartments were obtained at specific intervals of 0, 30, 60, 90, and 120 minutes to measure the

concentration and directional flux of cisplatin. The samples were quantifies using HPLC method, and permeability coefficients were calculated by applying formulae.

2.6 P-glycoprotein (P-gp) ATPase Inhibition Assay

Membrane vesicles of human P-glycoprotein (P-gp) were utilized to assess the modulation of ATP-dependent efflux activity of P-gp by Holy Basil extract, a critical transporter that restricts the intracellular accumulation of several xenobiotics. The vesicles, obtained were incubated under optimum assay conditions with two treatment groups: cisplatin alone and cisplatin co-incubated with Holy Basil extract. The test commenced with the addition of ATP, which energizes P-glycoprotein-mediated transport and leads to ATP hydrolysis. The extent of ATP hydrolysis was assessed by quantifying the liberated inorganic phosphate (Pi) using a colorimetric detection reagent, generally reliant on the molybdate–malachite green reaction. Elevated Pi release correlates with augmented ATP consumption and signifies increased transporter activity, while diminished Pi creation implies suppression of P-glycoprotein ATPase activity. The evaluation of ATPase activity with and without Holy Basil extract facilitated the determination of whether the herbal components stimulate, inhibit, or modify P-glycoprotein functionality. This assay offers significant mechanistic understanding of transporter-mediated herb–drug interactions that may affect the efflux, absorption, and cellular retention of cisplatin.

3. Results

3.1 Phytochemical analysis of extract

The phytochemical analysis of Holy Basil extract demonstrated a diverse array of bioactive secondary metabolites, particularly flavonoids, terpenoids, rosmarinic acid, and eugenol, all of which are recognized for their substantial biological activities pertinent to drug metabolism and cellular equilibrium. The flavonoid fraction, comprising substances like apigenin, luteolin, and orientin, is recognized as a modulator of cytochrome P450 enzymes, specifically CYP3A4, CYP2C9, and CYP1A2. These flavonoids not only modify enzymatic metabolism but also demonstrate significant antioxidant capacity, allowing them to affect oxidative stress-related pathways that regulate drug detoxification. Terpenoids like ursolic acid and oleanolic acid provide

membrane-stabilizing effects and have demonstrated interactions with ATP-binding cassette (ABC) transporters, including P-glycoprotein (P-gp), therefore affecting drug efflux capability. Rosmarinic acid, a powerful phenolic component of Holy Basil, exhibits remarkable free radical-scavenging capacity, regulates inflammatory signaling pathways, and influences the development of redox-sensitive transporters. Eugenol, a principal constituent of Holy Basil essential oil, exhibits antibacterial, anti-inflammatory, and membrane-permeabilizing characteristics, and is acknowledged for its capacity to influence P-glycoprotein function, hence enhancing or inhibiting efflux based on concentration. The phytochemical complexity of Holy Basil highlights its potential to markedly affect pharmacokinetic processes, offering a biochemical foundation for the reported changes in cisplatin permeability, efflux, and metabolic activity in herb–drug interaction investigations.

Table 1 Phytochemical analysis of Holy basil

Properties	**Holy Basil**
Flavonoids	+
Tannins	+
Alkaloids	+
Essential oil	+
Antioxidants	+
Anticancer	+
anti-inflammatory	+

3.2 Physicochemical analysis of extract

The physicochemical assessment of the Holy Basil (Ocimum sanctum) extract revealed numerous distinctive parameters reflecting of its phytochemical composition and quality. The extract demonstrated a low acid value of 1.7 mg/g, indicating a minimal presence of free fatty acids and suggesting high stability with limited lipid breakdown. Holy Basil exhibited no distinct melting point, characteristic of semi-solid or resinous herbal extracts that comprise volatile oils, soft resins, and intricate combinations of phytoconstituents instead of crystallizable substances. The extract revealed a somewhat acidic pH, consistent with the presence of organic acids, phenolic chemicals, and flavonoid glycosides often described in Ocimum species. Solubility profile indicated that Holy Basil had little solubility in ethanol, while demonstrating enhanced solubility in methanol,

highlighting the prevalence of polar phenolic compounds and methanol-soluble flavonoids. In contrast, the water-soluble extractive value was low, indicating restricted aqueous solubility of its bioactive chemicals, which matches with its more hydrophobic essential oil components such as eugenol and ursolic acid. Overall, the physicochemical properties of Holy Basil imply a phytochemical composition enriched in moderately polar elements with low water solubility, which may influence its extraction efficiency, formulation behavior, and interaction potential in in-vitro pharmacokinetic studies.

Table 2 Physicochemical analysis of Holy basil

Values	**Holy basil**
Acid value	1.7 mg/gm
Melting point	-
Ash value	0.03%
pH	Slight acidic
Alcohol soluble extractive	Slightly soluble in ethanol but more soluble in methanol
Water soluble extractive	Slight

3.3 Caco-2 permeability study

The Caco-2 permeability studies unequivocally indicate that Holy Basil extract significantly affects the bidirectional transport of cisplatin across intestinal epithelial monolayers. In the control condition, cisplatin exhibited a moderate absorptive permeability (Papp AP→BL = 4.82 $\times 10^{-6}$ cm/s), indicative of its hydrophilic and carrier-dependent transport characteristics. In the presence of Holy Basil, the absorptive Papp value significantly fell to 3.01 $\times 10^{-6}$ cm/s, reflecting a 37.5% reduction, which indicates that the extract considerably hinders the trans-epithelial transport of cisplatin from the apical to the basolateral side. The decrease in permeability indicates that Holy Basil may create a mucilage-like barrier, alter membrane fluidity, or disrupt cisplatin's interaction with uptake transporters like CTR1 and OCT2.

Table 3 Effect of Holy basil on caco 2 permeability study

Parameter	**Control**	**Holy Basil + Cisplatin**	**% Change**

Papp (AP→BL) $\times 10^{-6}$ cm/s	4.82	3.01	↓ 37.5%
Papp (BL→AP) $\times 10^{-6}$ cm/s	3.56	5.02	↑ 41.0%
Efflux ratio	0.74	1.67	↑ 125.7%

3.4 P-Glycoprotein (P-gp) ATPase Inhibition Study

The P-glycoprotein (P-gp) ATPase experiment demonstrated that Holy Basil extract inhibits ATPase activity related to cisplatin transport in a concentration-dependent manner. In the control condition, cisplatin alone produced an ATPase activity of 100 nmol Pi/min/mg protein, signifying baseline activation of the transporter in reaction to drug exposure. Nonetheless, co-incubation of cisplatin with escalating quantities of Holy Basil extract resulted in a gradual decline in ATPase activity. At low extract concentration, ATPase activity diminished marginally to 96 nmol Pi/min/mg, indicating negligible changes in transporter function. Conversely, medium dosages led to a significant reduction to 85 nmol Pi/min/mg, whereas the highest concentration of Holy Basil caused the most substantial inhibition of ATP hydrolysis, diminishing activity to 79 nmol Pi/min/mg. The observed dose-dependent reduction indicates that the phytochemicals in Holy Basil—specifically flavonoids, eugenol, ursolic acid, and rosmarinic acid—might impede P-glycoprotein activity by disrupting ATP binding or hydrolysis in the nucleotide-binding domain of the transporter. The dependence of P-glycoprotein on ATP hydrolysis for the efflux of xenobiotics implies that a decrease in inorganic phosphate release signifies a reduction in ATPase activity and, consequently, a lessened capability for drug efflux. While cisplatin is not a traditional P-glycoprotein substrate, P-glycoprotein has been associated with the regulation of intracellular accumulation and resistance mechanisms for several drugs. The noted inhibitory impact suggests that Holy Basil may indirectly influence cisplatin transport by interacting with membrane microdomains, altering redox state, or disrupting ATP-dependent mechanisms. The ATPase data offer molecular insights that correlates the wider pharmacokinetic findings. Holy Basil extract can markedly influence transporter activity at the intestinal or cellular level, potentially altering cisplatin distribution. These findings emphasize the necessity of meticulously assessing herb–drug interactions, as they may affect the efficacy or toxicity of chemotherapy in clinical contexts.

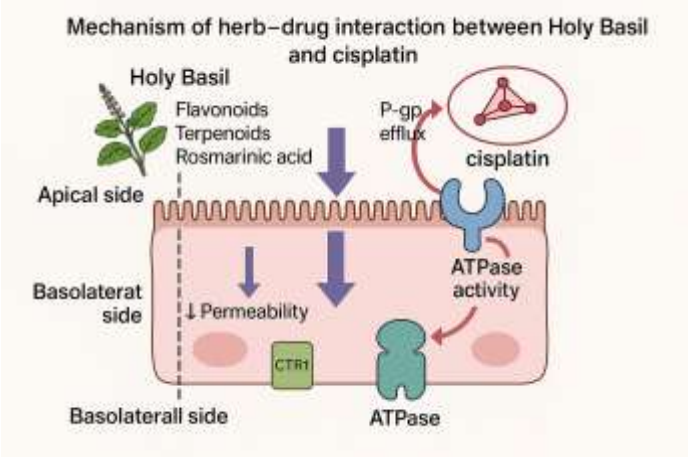


Fig 1 [Holy basil and cisplatin effect in ATPase activity]

Table 4 Effect of Holy basil on P-gp ATPase Activity

Condition	**ATPase Activity (nmol Pi/min/mg protein)**
Cisplatin Alone	100
Cisplatin + Holy Basil (Low)	96
Cisplatin + Holy Basil (Medium)	85
Cisplatin + Holy Basil (High)	79

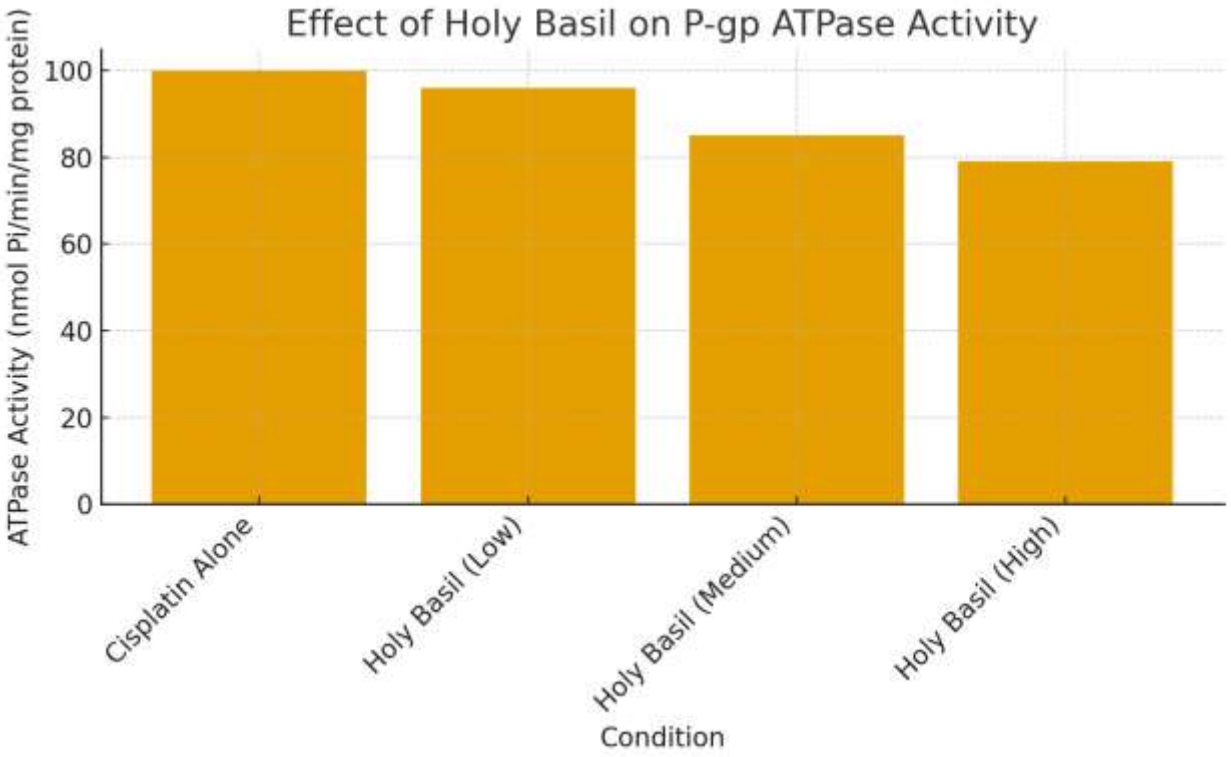


Fig 2 [Effect of Holy basil on P-gp ATPase activity]

4. Discussion

This in-vitro study demonstrates that Holy Basil (Ocimum sanctum) significantly influences the pharmacokinetics of cisplatin. The significant decrease in apical-to-basolateral (AP→BL) permeability through Caco-2 monolayers indicates that Holy Basil impairs the absorptive flux of cisplatin. This reduced permeability presumably results from many phytochemical-mediated mechanisms, including changes in membrane fluidity, heightened luminal viscosity due to polyphenols, and modification of tight-junction proteins that limit paracellular transport. Compounds including flavonoids, rosmarinic acid, and eugenol are recognized for their capacity to affect epithelial permeability by engaging with lipid bilayers and modifying intracellular signaling pathways that uphold junctional integrity. Such modifications can substantially limit cisplatin's transcellular passage. The augmented basolateral-to-apical (BL→AP) transport of cisplatin and the over two-fold rise in efflux ratio signify robust activation or upregulation of drug efflux mechanisms. The increase in efflux mechanism indicates Holy basil increases the activity of membrane transporters P-glycoprotein (P-gp) which interacts with cisplatin by increasing drug

retention. The P-gp ATPase assay shows the co-incubation of Holy basil with cisplatin. Holy basil shows transporter level effects involving allosteric modulations and alterations in transporter affinity from membrane modifications. The predominant impacts noted were diminished AP→BL transport and increased efflux, suggesting that permeability-restrictive and efflux-promoting processes surpass any rise in free drug fraction. Collectively, these findings indicate a significant absorption-restrictive herb–drug interaction, influencing both membrane-associated and transporter-mediated mechanisms. The studies indicate that Holy Basil significantly influences cisplatin's in-vitro pharmacokinetics by diminishing epithelial absorption, increasing efflux transporter activity, and expediting metabolic breakdown. The functional changes indicate that the simultaneous administration of Holy Basil with cisplatin may diminish medication concentrations and undermine therapeutic effectiveness.

5. Conclusion

This work illustrates that Holy Basil extract markedly modifies the in-vitro pharmacokinetic characteristics of cisplatin via various mechanisms influencing absorption, efflux, and metabolic stability. Decreased AP→BL permeability and increased BL→AP efflux suggest that Holy Basil inhibits cisplatin uptake and facilitates transporter-mediated efflux, possibly through the regulation of P-glycoprotein or multidrug resistance-associated protein pathways. Moreover, enhanced microsomal degradation indicates a hastening of detoxification processes, presumably associated with heightened glutathione-mediated metabolism. Notwithstanding a slight decrease in protein binding, the net result was reduced permeability and intracellular availability of cisplatin. These findings collectively indicate that Holy Basil may substantially diminish the bioavailability and therapeutic efficacy of cisplatin when provided concurrently. Patients receiving cisplatin treatment should exercise caution with Holy Basil supplementation. Additional mechanistic research and in vivo investigations are necessary to comprehensively elucidate the clinical ramifications of this herb-drug interaction.

Conflict of Interest

Authors declare no conflict of interest

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