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Original Article

ROLE OF P-GP INHIBITORS ON GUT PERMEATION OF METFORMIN: AN EX-VIVO STUDY

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ABSTRACT

Objective: Metformin hydrochloride is a biguanide derivative that is commonly used to treat type 2 diabetes. Metformin has a low oral bioavailability of 50% to 60 %. To overcome these challenges, metformin was used as a Pgp substrate in this research work and used in conjunction with natural P-gp inhibitors.

Methods: The study commenced with a chicken non-everted gut sac model that closely resembled *in vivo* intestinal transport processes. The effect of different P-gp inhibitors on Metformin intestinal permeability was examined in this study to fully recognize the potential significance of Pgp and intestinal metabolism.

Results: After evaluating the effectiveness of different P-gp inhibitors at different concentration concentrations i.e. Piperine, Ginger, Drumstick, and Verapamil (standard) at (2 mg/ml, 4 mg/ml, and 6 mg/ml) by non-everted gut sac study. At 2 mg/ml ginger and drumstick could not show any significant improvement. At 4 mg/ml also drumstick could not show any significant improvement in percentage drug permeation. At 6 mg/ml all three natural inhibitors show a significant difference in percentage drug permeation when compared using the *f2* similarity index. But piperine was found to be the most potent of all 3 inhibitors because it shows complete release with higher permeation in less time than ginger and drumstick when given in conjunction with Metformin. Then the comparative permeation study of different concentrations (i.e. 2 mg/ml, 4 mg/ml, and 6 mg/ml) of P-gp inhibitors was carried out using the *f2* similarity parameter and was that there is no significant difference in the percentage of drug permeation of Metformin in the presence of 2 mg/ml versus 4 mg/ml inhibitors. The same is with 4 mg/ml versus 6 mg/ml of inhibitors. However, when the percentage drug permeation of Metformin in the presence of 2 mg/ml as compared to 6 mg/ml, a significant difference was observed.

Conclusion: It was concluded from this research work that Piperine shows significant improvement in % drug permeation when compared using the *f*2 similarity index and its formulation with metformin may offer a simple and safe approach to enhance the pharmacological profile of metformin for effective anti-diabetic therapy in humans.

Keywords: P-glycoprotein, P-gp inhibitors, Drug efflux, Bioavailability, Absorption, Permeability

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INTRODUCTION

P-glycoprotein (P-gp) (P is permeability) is a member of the ATP-binding cassette (ABC) transporter superfamily that acts as a physiological barrier by removing harmful and foreign substances from cells. In humans, P-gp is a small gene family with two isoforms. The MDR1/ABCB1 isoform transports drugs, while the MDR2/3/ABCB4 isoform exports phosphatidylcholine into the bile [1]. P-gp was isolated in Colchicine-resistant Chinese hamster ovary cells in 1976, where it modified drug permeability and showed anticancer resistance [2]. The research data demonstrated that P-gp has the potential to develop resistance to cytotoxic agents. P-glycoprotein is present in cells other than tumors. It is also expressed in various normal, non-cancerous epithelial and endothelial cells, including the adrenal cortex, the brush border of the proximal renal tubule epithelium, the luminal surface of biliary hepatocytes, pancreatic ductules, and small and large intestine mucosa. The presence of p-glycoprotein in both the small and large intestines is particularly intriguing.

P-gp is unique in its potential to recognize substrate molecules and rapidly expel them from the gastrointestinal lumen, restricting absorption into the systemic circulation and actively enhancing excretion from the body via biliary and urinary routes. Such studies have facilitated research to inhibit P-gp activities in order to increase drug delivery and combat drug resistance. Distinct synthetic and natural origin compounds have shown the potential to inhibit P-gp transport activity, leading to increased intracellular drug accumulation, MDR reversal [3], and improvement of the pharmacokinetic and pharmacodynamic profiles of various challenging molecules, beneficial in designing clinically useful oral formulations of drugs that, due to poor oral absorption, are administered only via parenteral routes, also affect absorption, distribution, metabolism, and elimination of P-gp substrates in the process of improving pharmacokinetics.

Metformin hydrochloride is a biguanide derivative that is commonly used to treat type 2 diabetes. Metformin is absorbed primarily from the upper small intestine after oral administration and has poor bioavailability. Metformin has an absolute bioavailability of 50% to 60%. Its bioavailability problem is attributed to the presence of an intestinal efflux transporter, P-glycoprotein (P-gp), an ATP-binding protein [4]. It has a biological half-life ($t_{1/2}$) of 0.9–2.6h. For better treatment outcomes, high dosages of metformin (500 mg two or three times every day, or 850 mg either once or twice every day with or after meals) must be administered repeatedly. As a result, patient compliance decreases, and adverse effects such as nausea, anorexia, diarrhea, weight loss, vomiting, and taste disturbance become more common. Furthermore, biguanides have been associated with lactic acidosis, which can be lethal [5]. To overcome these challenges, metformin is used as a Pgp substrate in this research and can be used in conjunction with natural P-gp inhibitors.

During oral absorption, the rate and amount of drug diffused over the basolateral membrane to reach the systemic circulation are determined by drug characteristics (solubility and permeability) and P-gp efflux through the intestine apical membrane [6]. As a result, P-gp efflux screening is an essential phase in the drug development process. Sparreboom *et al.* [7], identified the function of P-gp in reducing Paclitaxel (PTX) oral absorption and facilitating direct excretion of the drug from the systemic circulation into the intestinal lumen. The results demonstrate that the oral bioavailability of Paclitaxel (PTX) increased from 11 % in wild-type mice to 35 % in

mdr1a (−/−) mice, while the cumulative fecal excretion decreased from 87 % of the given dosage in wild-type mice to 3 % in mdr1a (−/−) animals. As a result, P-gp efflux is characterized as one of the aspects of oral drug bioavailability and intestinal efflux [8]. Because oral administration is among the most significant and preferred routes of drug delivery, it is significant to overcome the absorption barrier imposed by P-gp.

For oral absorption analysis, the Caco-2 cell line is employed; however, it is very expensive and must be cultivated for many weeks before it can be used, as well as a cell-culture infrastructure is required. In general, drug transport over the layer mimics oral drug bioavailability in humans [9]; however, the rate of transport of molecules across the cell layer is extremely slow. Because it is widely assumed that the small intestine is the primary site of drug absorption for orally delivered pharmaceuticals, reliable ex-vivo research for examining drug transport across the small intestinal epithelium will be beneficial. The gut sac model has been widely studied for pharmacokinetic studies on drug absorption, drug metabolism or pro-drug conversion in gastrointestinal segments, efflux transport, multidrug resistance, and the effect of efflux transport modulators on drug absorption. The existence of a mucus layer and a relatively high surface area suitable for absorption are benefits of this model [10]. In this study, a chicken ileum gut sac from the intestine was used.

MATERIALS AND METHODS

Material

Metformin HCL was obtained from Aarti Drugs Limited, Sarigam, Gujarat; Verapamil was obtained from Aurore Pharmaceuticals Private Limited Hyderabad, Telangana, India, and piperine, drumstick, ginger were obtained in standard packs from the local market and other materials for buffer was obtained from S. D Fine Chem Ltd., Mumbai.

Method

Ex-vivo gut sac study (Non-everted)

To evaluate drug transport from the mucosal to the serosal surface, the ex-vivo sac method was utilized. In this research, a small portion of a non-everted intestinal sac of chicken was utilized to demonstrate the efflux mechanism of an anti-diabetic agent. The ileum was collected and sectioned off (10 cm each). The sections were then rinsed with a physiological solution (such as oxygenated tyrode's solution). The ileum was then clamped at 37 °C before being injected with a drug solution. The filled intestinal section was then closed by clamping the other end. The filled intestinal sac was placed in a beaker containing 200 ml oxygenated medium at 37 °C after another end was clamped. An oxygen supply was provided by the use of an aeration tube. The sampling was performed at different time intervals and analyzed using UV spectroscopy (Shimadzu) at 234 nm.

Fig. 1: Assembly of non-everted gut sac study

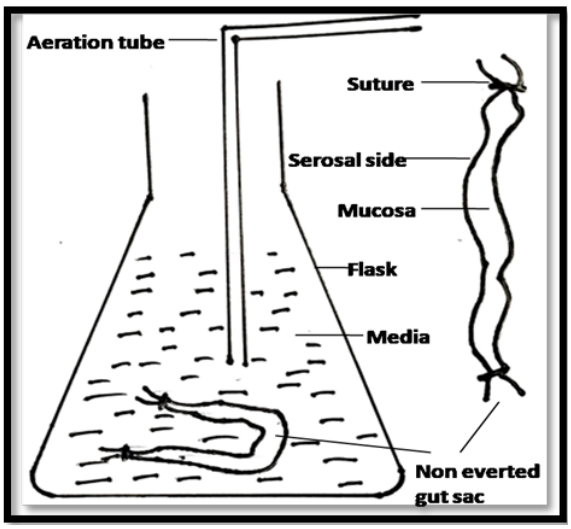

Fig. 2: Schematic diagram of non-everted gut sac model

Extraction method

- **Ginger**

Ginger rhizome was cleaned, rinsed under running water, sliced into little pieces, and dried in the open air. The powdered dried rhizomes were kept at lab temperature (20–23 °C). 0.2 g of this powder was macerated in 100 ml of distilled water at room temperature for 12 h before being filtered. The concentrate had a concentration of 2 mg/ml [11]. Different concentrations were prepared similarly.

- **Pepper**

Piperine was extracted from black pepper seeds utilizing ethanol as a solvent. For 2-3 h, powdered black pepper (0.2 g) was macerated to obtain in 100 ml of 20% ethanol at room temperature before being filtered [12]. The concentrate had a concentration of 2 mg/ml. Different concentrations were prepared similarly.

- **Drumstick**

Dried powder of pods of drumstick (Moringa oleifera) (0.2 g) was macerated with 100 ml of a hydroalcoholic solution containing 20% v/v ethanol for 24 h. The extract was then filtered and used [13].

RESULTS

Permeation study

Three natural inhibitors (piperine, ginger, and drumstick) and Verapamil (standard) were studied to check their P-gp inhibition capacity by non-everted gut sac study with drug concentration maintained at 2 mg/ml and percentage drug permeation studied. The data reveals that Verapamil and piperine show significant improvement in percentage drug permeation when compared using the *f2* similarity index. At the same time, ginger and drumstick could not show any significant improvement.

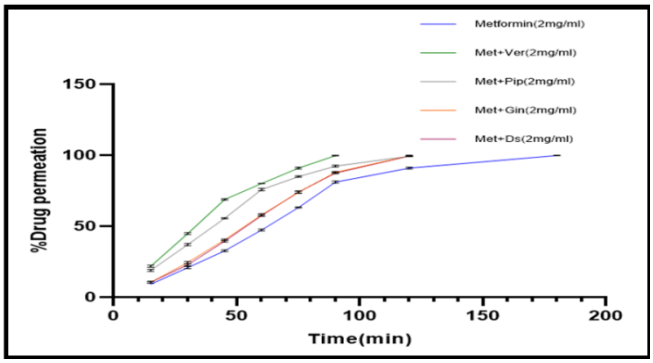

Fig. 3: Permeation study of metformin in the presence and absence of P-gp inhibitors at 2 mg/ml concentration (n=3, all data are in mean±SD)

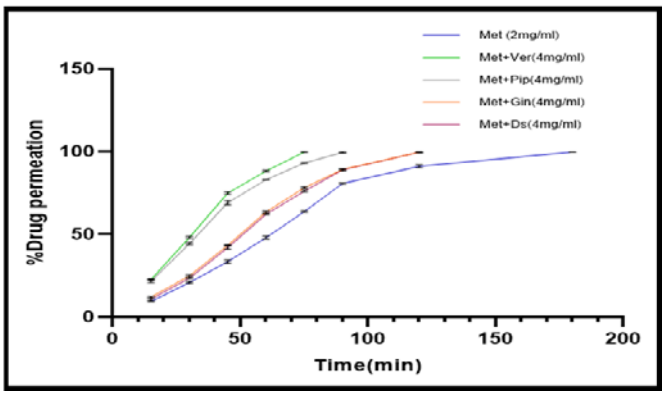

Fig. 4: Permeation study of metformin in the presence and absence of P-gp inhibitors at 4 mg/ml concentration (n=3, all data are in mean±SD)

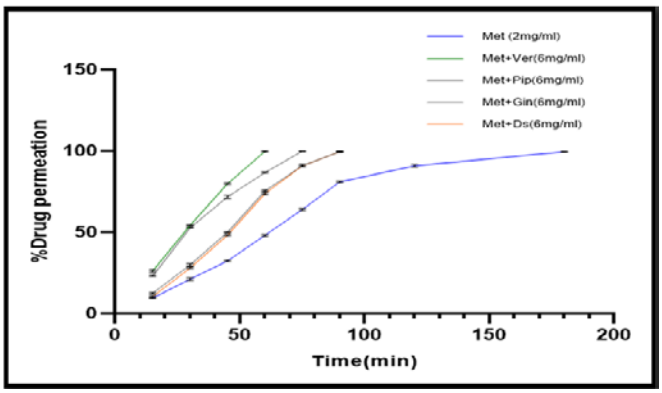

Fig. 5: Permeation study of metformin in the presence and absence of P-gp inhibitors at 6 mg/ml concentration (n=3, all data are in mean±SD)

Three natural inhibitors (piperine, ginger, and drumstick) and Verapamil (standard) were studied to check their P-gp inhibition capacity by non-everted gut sac study with drug concentration maintained at 2 mg/ml and percentage drug permeation studied. The data reveals that Verapamil and piperine and Verapamil and Ginger show significant improvement in percentage drug permeation when compared using the *f2* similarity index. At the same time, the drumstick could not show any significant improvement at this concentration.

Three natural inhibitors (piperine, ginger, and drumstick) and Verapamil (standard) were studied to check their P-gp inhibition capacity by non-everted gut sac study with drug concentration maintained at 2 mg/ml and percentage drug permeation studied. The data reveals that all three natural inhibitors show a significant difference in percentage drug permeation when compared using the *f2* similarity index. But Piperine was found to be the most potent of all 3 inhibitors because it shows complete release with higher permeation in less time than Ginger and Drumstick when given in conjunction with Metformin.

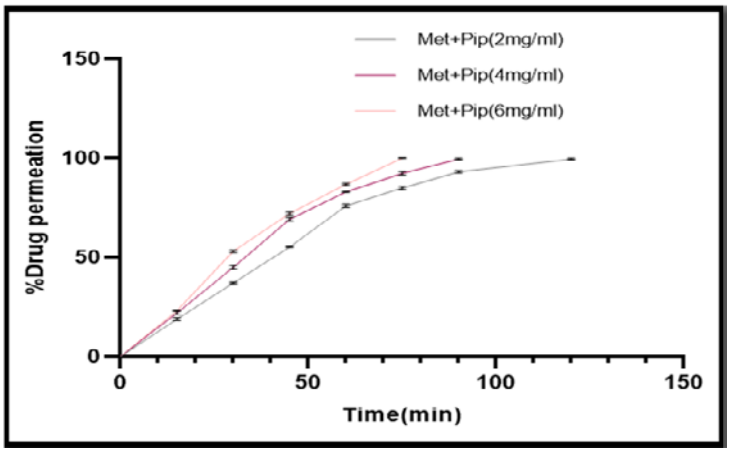

Fig. 6: Permeation study of metformin in the presence of piperine at 2, 4, and 6 mg/ml concentration (n=3, all data are in mean±SD)

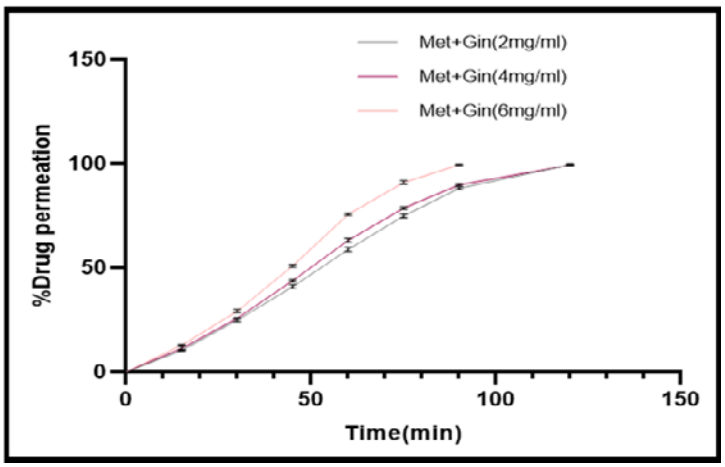

Fig. 7: Permeation study of metformin in the presence of ginger at 2, 4, and 6 mg/ml concentration (n=3, all data are in mean±SD)

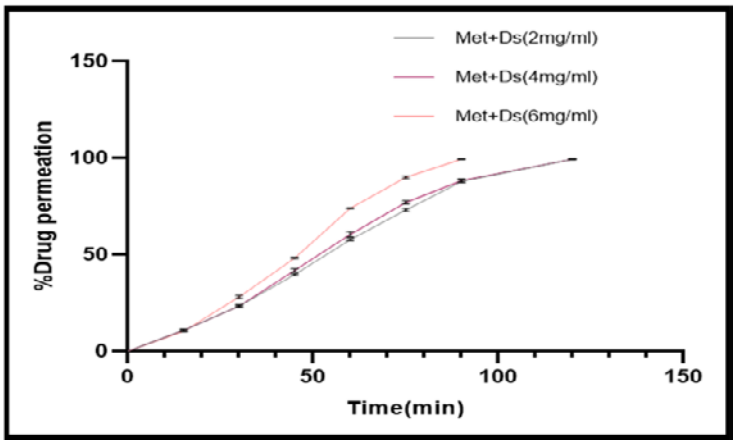

Fig. 8: Permeation study of metformin in the presence of drumstick at 2, 4, and 6 mg/ml concentration (n=3, all data are in mean±SD)

Comparative permeation study of different concentrations of P-gp inhibitors

It was observed in the above data, using the *f2* similarity parameter, that there is no significant difference in the percentage drug permeation of Metformin in the presence of 2 mg/ml of Piperine versus 4 mg/ml piperine. The same is with 4 mg/ml of piperine versus 6 mg/ml of piperine.

However, when the percentage drug permeation of Metformin in the presence of 2 mg/ml piperine was compared to 6 mg/ml piperine, a significant difference was observed.

It was observed in the above data, using the *f2* similarity parameter, that there is no significant difference in the percentage drug permeation of Metformin in the presence of 2 mg/ml of ginger versus 4 mg/ml of ginger. The same is with 4 mg/ml of ginger versus 6 mg/ml of ginger. However, when the percentage of drug permeation of Metformin in the presence of 2 mg/ml of ginger was compared with 6 mg/ml of ginger, a significant difference was observed.

It was observed in the above data, using the *f2* similarity parameter, that there is no significant difference in the percentage drug permeation of Metformin in the presence of 2 mg/ml of drumstick versus 4 mg/ml ginger. The same is with 4 mg/ml of drumstick versus 6 mg/ml of a drumstick. However, when the percentage drug permeation of Metformin in the presence of 2 mg/ml of drumstick was compared with 6 mg/ml drumstick, a significant difference was observed.

DISCUSSION

This research work was carried out to determine the efficacy of co-administered P-gp inhibitors on Metformin permeation. The study commenced with a chicken non-everted gut sac model that closely resembled *in vivo* intestinal transport processes. The effect of different P-gp inhibitors on Metformin intestinal permeability was examined in this study to fully recognize the potential significance of P-gp and intestinal metabolism. Bano G., Amla V., *et al.*, 1987 investigated the modulatory effects of phytochemicals on P-gp *in vivo* using doxorubicin as a model P-gp substrate. Piperine and capsaicin affected the pharmacokinetics and tissue distribution of doxorubicin *in vivo*, according to the findings. Thus, inhibiting P-gp with piperine or capsaicin can lead to a decrease in doxorubicin excretion into bile and urine, resulting in doxorubicin deposition in these regions. Amongst the phytochemicals investigated, capsaicin appears to be a potential agent for delivering P-gp substrates into target tissues or reversing MDR in cancer. Phytochemicals may potentially be effective in inducing food-drug interactions as a technique for increasing the bioavailability or prolonging the efficacy of P-gp substrate drugs [14].

Piperine, a key ingredient of black and long pepper, was found as an inhibitor of both human P-glycoprotein and CYP3A4 by Zutshi *et al.*, 1985. Piperine reduced the transfer of digoxin and cyclosporine A, both P-glycoprotein substrates. Second, the administration of black pepper (1 g, single dose) or piperine (single or repeated doses) increased plasma concentrations of the P-glycoprotein substrates phenytoin and rifampin by about 2-fold. In conclusion, they show that a significant component of pepper suppresses the activity of human P-glycoprotein [15]. So for this research work, piperine was taken as an inhibitor along with other natural P-gp inhibitors by taking Metformin as a model drug and evaluating its effect by a non-everted gut sac model.

Out of the three P-gp inhibitors used for this study, including piperine, ginger, and drumstick. Piperine significantly increased Metformin permeability in non-everted gut sac tissue, resembling *in vivo* intestinal transport processes. It is assumed that the increase in permeability of Metformin at 6 mg/ml concentration of piperine is due to significant inhibition of P-gp-mediated efflux. The extent of the bioenhancement of Metformin achieved with piperine in the current study suggests that piperine should be investigated further in the *in vivo* study for its pharmacokinetic enhancing effects. According to the study's outcomes, piperine can increase Metformin absorption by blocking P-glycoprotein in a concentration-dependent manner.

CONCLUSION

The goal of this study was to investigate if co-administered P-gp inhibitors, piperine, ginger, and drumstick, affected Metformin absorption in an ex-vivo chicken non-everted gastrointestinal sac model. In the present research, piperine significantly improved drug permeation when compared to ginger and drumstick. Piperine at 6 mg/ml has been shown to substantially block the Pgp efflux pump and increase Metformin permeability. The present study demonstrates that piperine in a sufficient proportion can be extremely beneficial in enhancing Metformin oral bioavailability. This method might provide a straightforward and safe way to improve Metformin's pharmacological profile for successful anti-diabetic therapy in humans.

LIST OF ABBREVIATIONS

P-gp: P-glycoprotein, ABC: ATP-binding cassette, Met: Metformin, Ver: Verapamil, Pip: Piperine, Gin: Ginger, Ds: Drumstick

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Isha Shah prepared the manuscript, Nensi Raytthatha helped in the compilation of data, and Jigar Vyas and Umesh Upadhyay guided for preparation of the manuscript. All authors read and made corrections to the finalized manuscript before submission.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Sharom FJ. The P-glycoprotein multidrug transporter. Essays Biochem. 2011 Sep;50(1):161-78. doi: 10.1042/bse0500161, PMID 21967057.
2. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta. 1976 Nov;455(1):152-62. doi: 10.1016/0005-2736(76)90160-7, PMID 990323.
3. Shukla S, Wu CP, Ambudkar SV. Development of inhibitors of ATP-binding cassette drug transporters: present status and challenges. Expert Opin Drug Metab Toxicol. 2008;4(2):205-23. doi: 10.1517/17425255.4.2.205. PMID 18248313.
4. Loo TW, Bartlett MC, Clarke DM. Identification of residues in the drug translocation pathway of the human multidrug resistance P-glycoprotein by arginine mutagenesis. J Biol Chem. 2009 Sep 4;284(36):24074-87. doi: 10.1074/jbc.M109.023267, PMID 19581304.
5. O'Rourke A. Martindale: The complete drug reference. American Journal of Health-System Pharmacy. 2010;67(4):321. doi: 10.2146/br090004.
6. Hoosain FG, Choonara YE, Tomar LK, Kumar P, Tyagi C, du Toit LC. Bypassing P-glycoprotein drug efflux mechanisms: possible applications in pharmacoresistant schizophrenia therapy. BioMed Res Int. 2015;2015:484963. doi: 10.1155/2015/484963. PMID 26491671.
7. Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA. 1997 Mar 4;94(5):2031-35. doi: 10.1073/pnas.94.5.2031, PMID 9050899.
8. Takano M, Yumoto R, Murakami T. Expression and function of efflux drug transporters in the intestine. Pharmacol Ther. 2006 Jan;109(1-2):137-61. doi: 10.1016/j.pharmthera.2005.06.005, PMID 16209890.
9. Artursson P, Karlsson J. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem Biophys Res Commun. 1991 Mar 29;175(3):880-5. doi: 10.1016/0006-291x(91)91647-u, PMID 1673839.
10. Barthe L, Woodley JF, Kenworthy S, Houin G. An improved everted gut sac as a simple and accurate technique to measure paracellular transport across the small intestine. Eur J Drug Metab Pharmacokinet. 1998 Apr-Jun;23(2):313-23. doi: 10.1007/BF03189357, PMID 9725499.

11. Kamtchouing P, Mbongue Fandio GY, Dimo T, Jatsa HB. Evaluation of the androgenic activity of zingiber officinale and pentadiplandra brazzeana in male rats. Asian J Androl. 2002 Dec;4(4):299-301. PMID 12508133.
12. Sreevidya N, Mehrotra S. Spectrophotometric method for estimation of alkaloids precipitable with dragendorff's reagent in plant materials. J AOAC Int. 2003 Nov-Dec;86(6):1124-7. doi: 10.1093/jaoac/86.6.1124, PMID 14979692.
13. Sadek KM. Chemotherapeutic efficacy of an ethanolic Moringa oleifera leaf extract against chromium-induced testicular toxicity in rats. Andrologia. 2014 Nov 12;46(9):1047-54. doi: 10.1111/and.12196, PMID 24215114.
14. Bano G, Amla V, Raina RK, Zutshi U, Chopra CL. The Effect of piperine on the pharmacokinetics of phenytoin in healthy volunteers. Planta Med. 1987 Dec;53(6):568-9. doi: 10.1055/s-2006-962814, PMID 3444866.
15. Zutshi RK, Singh R, Zutshi U, Johri RK, Atal CK. Influence of piperine on rifampicin blood levels in patients of pulmonary tuberculosis. J Assoc Physicians India. 1985 Mar;33(3):223-4. PMID 4044481.