Chapter 7

Summary & Conclusion
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Food and its components interact with co-administered drugs and affect their availability across intestine. Several pharmacokinetic parameters are used to determine their effect on bioavailability of co-administered drugs. There has been the practice of using some substances in the formulations of complementary or traditional medicine that have the bioavailability enhancing effect on drugs. These practices include the concept of Trikatu, i.e., three acrids consisting of long pepper, black pepper and dry ginger used in equal proportions in combination or individually.

The present work was designed to provide bioavailability enhancers and to characterize active fractions phytochemically. Based on the literature reports *Camellia sinensis* (L.) Kuntze leaves, *Nigella sativa* Linn. seeds and *Piper longum* Linn. fruits were selected for the present research.

The methanolic and hexane extracts of selected plants were evaluated for *in-vitro* bioavailability enhancement studies of a model drug Amoxicillin. Everted rat intestinal sacs were used for *in-vitro* experiment to study the transfer of Amoxicillin across the gut. Amoxicillin (6 mg/ml) was co-infused with 3 mg and 6 mg of methanol and hexane extracts of plants separately. The amount of Amoxicillin that traversed the gut was followed spectrophotometrically at 273 nm and 254 nm. The results indicated that the methanolic extract of all three plants interacted with the co-infused Amoxicillin and significantly increased its penetration across gut wall in the order *N. sativa* > *P. longum* > *C. sinensis*.

The comparative *in-vitro* studies revealed that methanol and hexane extracts of *N. sativa* increased the permeation of Amoxicillin significantly (*P* < 0.001) as compared to control. Permeation was also found to be significantly higher for the hexane extract (*P* < 0.001) in comparison to methanol extract at the same dose levels (I). Oleic acid, an unsaturated fatty acid present in the hexane extract of *N. sativa* seeds, was also tested at concentrations 0.6 mg and 1.2 mg (equivalent to 3 mg and 6 mg of hexane extract, respectively). It was observed that the permeation of Amoxicillin with 0.6 mg oleic acid increased significantly (*P* < 0.001) compared to control. It was found to be equivalent to permeation enhancement with hexane extract (3 mg). Contrary to hexane extract, oleic acid did not show a dose-dependent increase in permeation of Amoxicillin across
(glyceryl-1-octadec-9'-enoyl-2-octadecanooate); ethyl oleate (ethyl octadec-9'-enoate),
were isolated Decanyl nigelloic acid diglucoside (α-decanyl-3' aldehydic-4-methoxy-5-
hydroxy benzoyl-5-β-D-glucofuranonyl (2→1)-β-D-glucopyranosyl (2→1)-β-D-
glucopyranoside) and nigelabdienoyl triglucoside (homolabd- 5, 9 (11) -dien-16-onyl-β-
D-glucopyranosyl (2→1)-β-D-glucopyranosyl (2→1)-β-D- glucopyranoside) are being
reported for the first time from N. sativa.

The phytochemical characterization of bioenhancing methanolic fraction of P. longum
led to the isolation of piperlongum flavanoyl sesterterpeonate (5, 7-dihydroxy-4'-methoxy
flavanonyl-7-(11E, 15E)-2', 10', 14', 18'-tetramethyl-10'-hydroxy-6''-
hydroxymethylene eicos-11'''), 15''-dien-1-oate); longumyl ethylamide (1-[2(E, 14Z)-15-
(3', 4'-benzodioxol-1'-yl)-pentadec-2, 14-dienoyl] ethylamide); naphthaquinoyl
dodecanoyl amidodiglycosyl laurate (1-[2(E, 11E)-12-(18-hydroxy-14, 21-
naphthalenedionyl)-dodec-2, 11-dien-4, 10-dioxo-1-onyl]-pentyl amido-18-O-β-D-
galactofuranosyl (6→1)- O-β-D-glucopyranosyl-2''''-dodecanooate); longumyl diglucosyl
hexacosenoate (1 - [(2E)-9-(12,13- dihydroxyphenyl)-non-2-en-1-oyl]-pentylamido- 13 -
O - β - D - glucopyranosyl (2→1) - O - β - D - glucopyranosyl- 2''''-hexacos-9,12-
dienoate); longumyl heptamido glucosyl myristate (1-[2(E)-7-(10, 11-dihydroxyphenyl)-
hept-2-en-1-oyl]-pentylamido-11-O-β-D-glucopyranosyl-6'''-tetradecan-1-oate);

hexacosanoic acid xyloside (n-hexacosanoyl-β-D-xylofuranoside); dehydrodregneric
acid diglucoside (n-tetracos-9-enoyl-β-D-glucopyranosyl (2→1)-O-β-D-
glucopyranoside); piperlongumoic acid dixyloside (3-methoxy-4, 5-dihydroxy phenyl
acetic acid-5-O-β-xylofuranosyl (2→1)-O-β-xylofuranoside); butanoic acid
tetraglycoside (n-butanoyl-β-D-galacto- furanosyl (2→1)-O-β-D-glucopyranosyl (2→1)-
O-β-D-glucopyranosyl (2→1)-O-β-D-glucopyranoside) and butanoic acid diglucoside (n-
butonyl -β-galactofuranosyl (2→1)-O-β-D-glucopyranoside). A total of ten compounds
were isolated of which seven compounds were isolated for the first time from this plant.

A GC-MS analysis of bioenhancing hexane extract of N. sativa seeds showed the
presence of thirty-two fatty acids, which represented 99.9% of total composition. It
contained fifteen saturated fatty acids (17%) and seventeen unsaturated fatty acids
(82.9%). Linoleic acid (50.2%), oleic acid (19.9%), margaric acid (10.3%), cis-11, 14-
eicosadienoic acid (7.7%) and stearic acid (2.5%) were the major components (III). The
fatty acid composition was similar to earlier reports. Linoleic acid and oleic acid along with other unsaturated fatty acids have been reported to be responsible for bioenhancement of many drugs in the past. It was thus postulated that since the hexane extract of *N. sativa* contains different types of fatty acids, a synergistic effect was evident.

Having its highest effect on permeation of Amoxicillin, the hexane extract *N. sativa* and oleic acid were selected for *in-vivo* bioenhancement studies. Adult male Wistar albino rats (200-250 g) were used for the experiments. Animals were kept under standard laboratory conditions during the experiments. The rats were fasted overnight before the day of the experiment. Animals were randomly distributed in three groups (*n* = 6). One group received Amoxicillin (25 mg/kg BW, p.o.), second group received Amoxicillin (25 mg/kg BW, p.o.) and *N. sativa* hexane extract (25 mg/kg BW, p.o.) while as the third group received Amoxicillin (25 mg/kg BW, p.o.) and oleic acid (5 mg/kg BW, p.o.). Blood samples were collected from tail vein in pre-heparinized glass tubes at different time intervals post-dosing (0, 0.250, 0.500, 0.750, 1, 1.5, 2, 4, 6 and 8 h). Blood samples were centrifuged (4000 rpm, 10 min, 4°C) to separate plasma. The amount of Amoxicillin in rat plasma was determined by UPLC-MS/MS method.

In the light of the diversity of analysis methods available, it was found prudent to develop and validate an in-house analysis method that can be successfully applied for *in-vivo* bioenhancement studies samples. A rapid, selective and sensitive method was developed and validated for Amoxicillin quantification in plasma by UPLC-MS/MS method. Sample preparation involved protein precipitation by acetonitrile followed by liquid-liquid extraction (LLE) with dichloromethane. Separation was performed on Aquity BEH C_{18} column using acetonitrile-1 mM ammonium acetate (85:15, v/v) as mobile phase. Analyte was detected by electro spray ionization mass spectrometry in positive ion multiple reaction monitoring mode. CCs with good linearity having *R* = 0.995 was obtained in the range of 100-20000 ng/ml. The extraction recovery was 88.87% that was significantly better than earlier reported methods. The method was validated in terms of selectivity, linearity, precision, accuracy, LOD, LOQ and stability of the analytes.
The validated UPLC-MS/MS was utilized for the determination of Amoxicillin in rat plasma samples in bioenhancement studies. The pharmacokinetic data revealed that hexane extract enhanced the $C_{\text{max}}$ and AUC of co-administered Amoxicillin. The $C_{\text{max}}$ enhanced from 4138.251 ±156.93 ng/ml to 5995.045 ±196.28 ng/ml while as AUC$_{0-t}$ was significantly increased from 8871.442 ± 143.33 ng.h/ml to 13428.585 ± 152.45 ng.h/ml (I). The percent relative bioavailability of Amoxicillin administered orally in combination with hexane extract was found to be 1.51 times higher than when administered alone. It indicated that *Nigella* hexane extract increased both the rate and extent of absorption of Amoxicillin. Oleic acid also increased $C_{\text{max}}$ and AUC of coadministered Amoxicillin in comparison to control but insignificantly. Fatty acids have been reported to act as bioenhancer by increasing the fluidity of apical and basolateral membrane. The higher bioenhancement with *N. sativa* hexane extract compared to oleic acid can be attributed to synergism shown by combination of fatty acids in the hexane extract.

In conclusion the results indicated that *N. sativa* hexane extract interacted with the co-administered Amoxicillin by increasing its absorption across gut wall. Thus *N. sativa* hexane extract was found to be efficient absorption enhancer and can be the part of bioavailability enhancing systems for various low permeable drugs.