Chapter 5

Summary
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Black and long peppers are being widely used as spice since time immemorial. In addition to their culinary use, they are a common ingredient of a large number of traditional medicines indicated for a wide spectrum of ailments. Piperine, an alkaloid isolated from peppers has been identified as the single molecule to which most of the biological effects of peppers could be attributed. The most important action of piperine reported so far is the enhancement of bioavailability of drugs. The work embodied in this thesis is an effort to further understand the role of this molecule as a bioavailability enhancer of drugs.

Effect of piperine on gastrointestinal tract

Gastrointestinal events such as gastric emptying and gastrointestinal transit play an important role in the oral bioavailability of drugs. Effect of piperine on gastric emptying has not been studied so far and its gastrointestinal transit inhibitory activity is reported at only one dose. During the present investigations, it was found that piperine inhibits gastric emptying of solids and liquids in rats. The studies also confirmed its gastrointestinal transit inhibitory activity. Dose and time dependence of both the activities was also revealed. Regarding the metabolites of piperine (piperic acid, piperonylic acid, piperonal and vanillic acid), only piperic acid was found to be active with higher inhibitory activity on gastric emptying of liquids as compared to piperine.

Development of new HPLC method for analysis of piperine in rat plasma

Even after a volume of research work done on piperine, surprisingly a HPLC method for its determination in plasma was not reported. Hence, a rapid, sensitive
and simple HPLC method for analysis of piperine in rat plasma was developed and used for studying the pharmacokinetics of piperine in rats. This method could be easily applied to the analysis of piperine in plasma/serum of other species owing to good separation from endogenous plasma components.

**Formulation dependent pharmacokinetics and biliary excretion of piperine in rats**

Complete plasma concentration-time profile of piperine was studied using solution and suspension formulations. It was demonstrated that suspension formulation of piperine results in poor oral bioavailability as compared to solution formulation. Solution formulation results in rapid absorption. Neither piperine nor piperic acid was detected in bile upto 6 h which contradicts previous reports. However, few new peaks were observed in bile which probably are metabolites of piperine other than piperic acid. Detection of piperine in faeces by some scientists could be due to the incomplete absorption and not biliary excretion as they used suspension formulation of piperine which results in poor oral bioavailability.

**Studies on metabolism of piperine in rats**

Earlier studies on the metabolism of piperine in rats reported four metabolites (piperonal, piperonylic acid, piperonyl alcohol and vanillic acid) in urine and one in bile (piperic acid). Three metabolites have also been reported in human urine which are structurally different from those reported in rats. A new metabolite of piperine has been characterised from rat urine during the course of present work. However, the present studies in rats failed to detect any of the previously reported metabolites in urine or bile. The new minor urinary metabolite was detected and characterised by LC/MS/MS studies. The structure of the
metabolite is proposed to be \( \text{E,E-1-[5-(3-methoxy-4-hydroxy-phenyl)-1oxo-2,4-pentadienyl]} \) piperidine formed by cleavage of the methylenedioxy ring of piperine.

![Piperine and Metabolite structures](image)

**Studies on bioavailability enhancement potential of piperine**

In order to further study the bioavailability enhancement effect of piperine, anticancer (5-fluorouracil), antiviral (Azidothymidine) and antibiotic (Cefixime) drugs were selected. Piperine was found to significantly increase the bioavailability of 5-fluorouracil and azidothymidine possibly due to inhibition of respective metabolising enzymes viz. dihydropyrimidine dehydrogenase and UDP-glucuronyltransferase. Cefixime was selected for specifically studying the effect of piperine on absorption and excretion as cefixime does not undergo biotransformation. However, piperine failed to show any effect on cefixime bioavailability. Multiple or single dose administration of piperine produces similar results. Metabolites of piperine viz. piperonal and piperonylic acid were unable to increase the bioavailability of any of the selected drugs suggesting the importance of side chain in the structure of piperine. Piperine showed *in-vitro* cytotoxicity and also increased the cytotoxicity of 5-fluorouracil against breast cancer cell lines indicating that it has potential not only to enhance the bioavailability but possibly potentiate the pharmacological effect of drugs also.

In conclusion, piperine appears to be a molecule with vast potential to increase the therapeutic utility or potency of many drugs with a strong possibility of reduction in their doses.