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Docking studies of piperine - iron conjugate with human CYP450 3A4

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Abstract:
Piperine, a major constituent of Piper nigrum (Black pepper), is one of the well known components in many Ayurvedic formulations. Piperine is most studied bioenhancer because it inhibits drug metabolizing enzymes in rodents and increases plasma concentrations of several drugs, including P-glycoprotein substrates. However, there areno evidences on piperine-iron conjugate to inhibit human CYP450 3A4. We therefore investigated the influence of piperine-Fe conjugate to study the metabolism of iron with CYP450 3A4. Our in silico results showed that Piperine when conjugated with iron, inhibited activity of CYP450 3A4. This improved the binding of piperine-Fe conjugate with CYP450 3A4 and increased bioavailability.

Key words: Bioavailability, metabolic enzymes, Cytochrome P450 isoenzymes, piperine-iron conjugate and docking scores.

Background:
Around 30 per cent of newly born babies in India suffer from acute iron deficiency caused due to malnourished mothers who also suffer from the same problem **[1-7].** The growing malnutrition problem in largely due to the dramatic change in food habits involving increasing shift from iron and micro-nutrient food to high energy and high fat fast food **[8-9]**. Black pepper (*Piper nigrum*) is one of the most widely used among spices. It is valued for its distinct biting quality attributed to piperine and its isomers. Black pepper is used not only in human dietaries but also for other purposes such as medicinal, as a preservative, in perfumery, even as insecticide. Like Piperine, curcumin could modulate P-glycoprotein and CYP3A4 expression, and in turn modify the pharmacokinetic profiles of P-glycoprotein and CYP3A4 substrates in male Sprague-Dawley rats. Curcumin also attenuated the CYP3A4 level in the small intestine but induced CYP3A4 expression in the liver and kidney **[10-12].** However, piperine inhibits both the drug transporter P-glycoprotein and the major drug-metabolizing enzyme CYP3A4. Because both proteins are expressed in enterocytes and hepatocytes and contribute to a major extent to first-pass elimination of many drugs, which indicate that dietary piperine could affect plasma concentrations of P-glycoprotein and CYP3A4 substrates in humans, in particular if these drugs are administered orally **[13].** Very recently, it is shown that a single administration of 1g of black pepper more than doubled area under the plasma concentration time curve and elimination half-life of phenytoin **[14].** In this paper we discussed the in silico docking studies of piperine conjugated with iron (Fe^{3+}) into Cytochrome P450 3A4 (CYP450 3A4). This implies the efficacy of conjugate on iron metabolism using cytochrome P450 red-ox system.

Methodology:
Tools employed
Protein Data Bank server (PDB:www.rcsb.org/pdb) **[15],** WhatIf server (http://swift.cmbi.ru.nl/servers /html/index.html) **[16],** ACDChemSketchand MoleGro Virtual Docker and Viewer Preparation of CYP450 3A4 and Piperine-Fe conjugate Cytochrome P450 3A4 structure was downloaded from PDB

server. The ID generated was 1W0E. The protein was optimized using Whatif server. The optimized protein was used for further analysis. Piperine-Fe conjugate structure was constructed using ACDChemSketch 12.01 software. The three dimensional structure of the Piperine-Fe conjugate was optimized using ACDChemSketch – Tools- - 3D structure optimization wizard. Docking studies of Piperine-Fe conjugate with 1W0E. The protein was imported into MoleGro Virtual Docker version 4.0.2.0 and surface was created. Cavities were detected in the protein surface. Five cavities were found and they were represented in green color. Piperine-Fe conjugate was imported into MoleGro Virtual Docker software in .mol format. This ligand was docked into cavities and it produced five docking sites with different amino acid sequences. The MolDock score and RMSD values were calculated.

Discussion:

Optimization of CYP450 3A4 and Piperine-Fe conjugate

The optimized structure of CYP450 3A4 (PDB ID: 1W0E) was shown in **Figure 1A.** The protein was subjected to detect cavities using MoleGro Virtual Docker version 4.0.2.0. The cavities were shown in green in **(Figure 1A**). Five cavities were detected as shown in **Figure 1A**. Piperine-Fe conjugate was optimized using ACDChemSketch 12.01. The optimized structure was shown in **Figure 1B.** Docking in each cavity generated five poses (Pose 1 through 5). The five poses generated are shown in **Figure 1B.** It should be noted that each pose has its own spatial arrangement.

Docking studies of Piperine-Fe conjugate with CYP450 3A4

Docking of piperine-Fe conjugate with cavities of CYP450 3A4 generated five poses with unique chemical arrangement. The cavity structures were shown in **Figure 2A, 2D & 2G**. **Figure 2B** represents pose-1 docked onto **Figure 2A, Figure 2C** represents pose-2 docked onto **Figure 2A, Figure 2E** represents pose-3 docked onto **Figure 2D, Figure 2F** represents pose-4 docked onto **Figure 2D, Figure 2H** represents pose-5 docked onto **Figure 2G**. All five poses were represented in **Figure 2I**.

Docking results were shown in **Table 1 (see supplementary material).** The MolDock Score and cavity volume of Pose 1 was high; the structure in the pose 1 would be superior to other poses. This implies that the structure in pose 1, was firmly bound with CYP450 3A4, making the protein more inactive. This leads to the reduction or inhibition of metabolism of Piperine-Fe conjugate. A similar report was also shown with crude piperine extract. To increase bioavailability of iron, we prepared Piperine-Fe conjugate and this ligand was docked with CYP450 3A4. This report suggests that conjugating piperine with iron may slow down the metabolism of iron, thereby piperine probably enhances the bioavailability of iron.

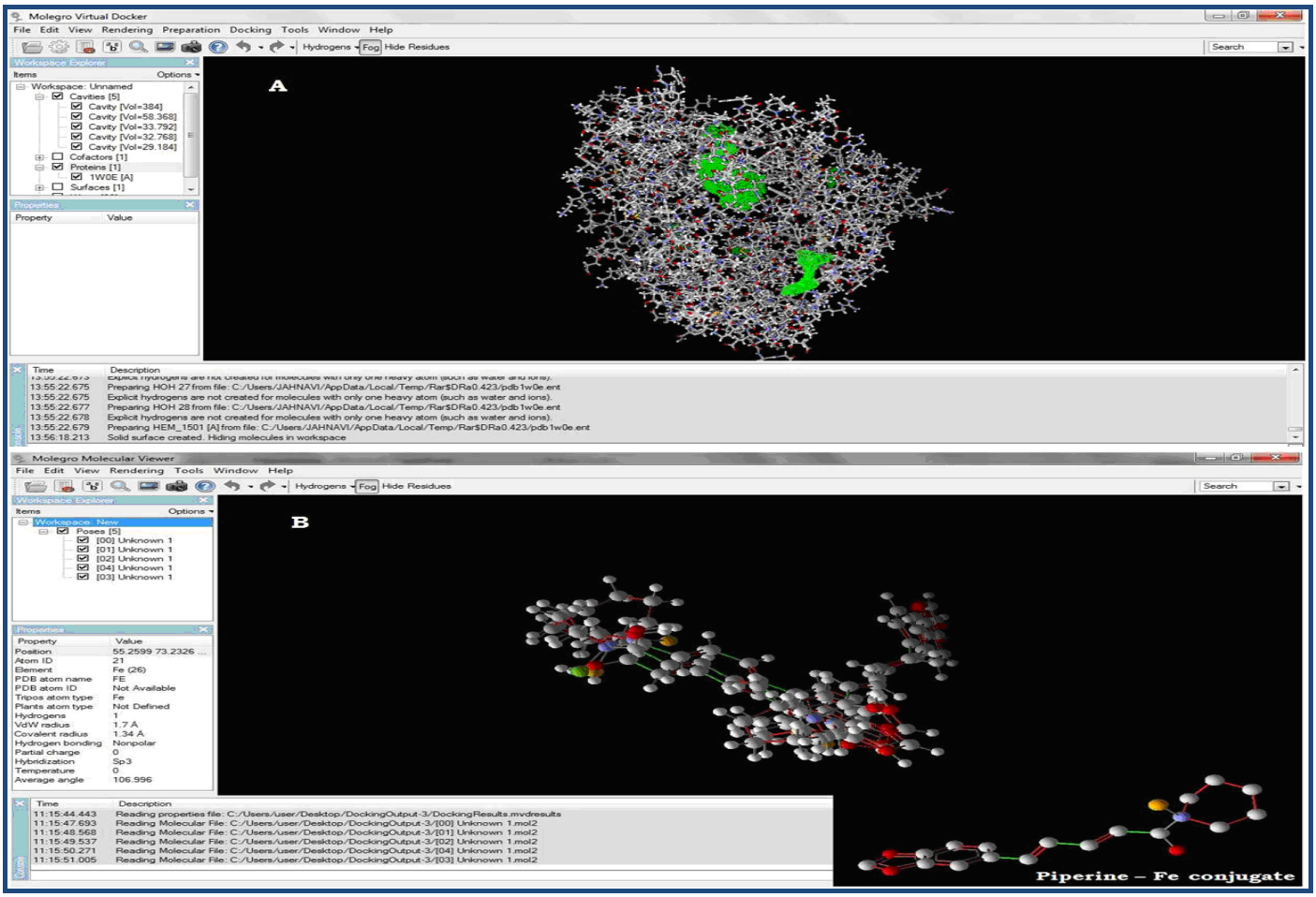

Figure 1: A) represents the cavities with protein structure. Green colored cavities are embedded in protein structure; **B**) represents optimized structures of piperine – iron conjugates. All five structures of Piperine-Fe conjugates are given in Figure 1B.

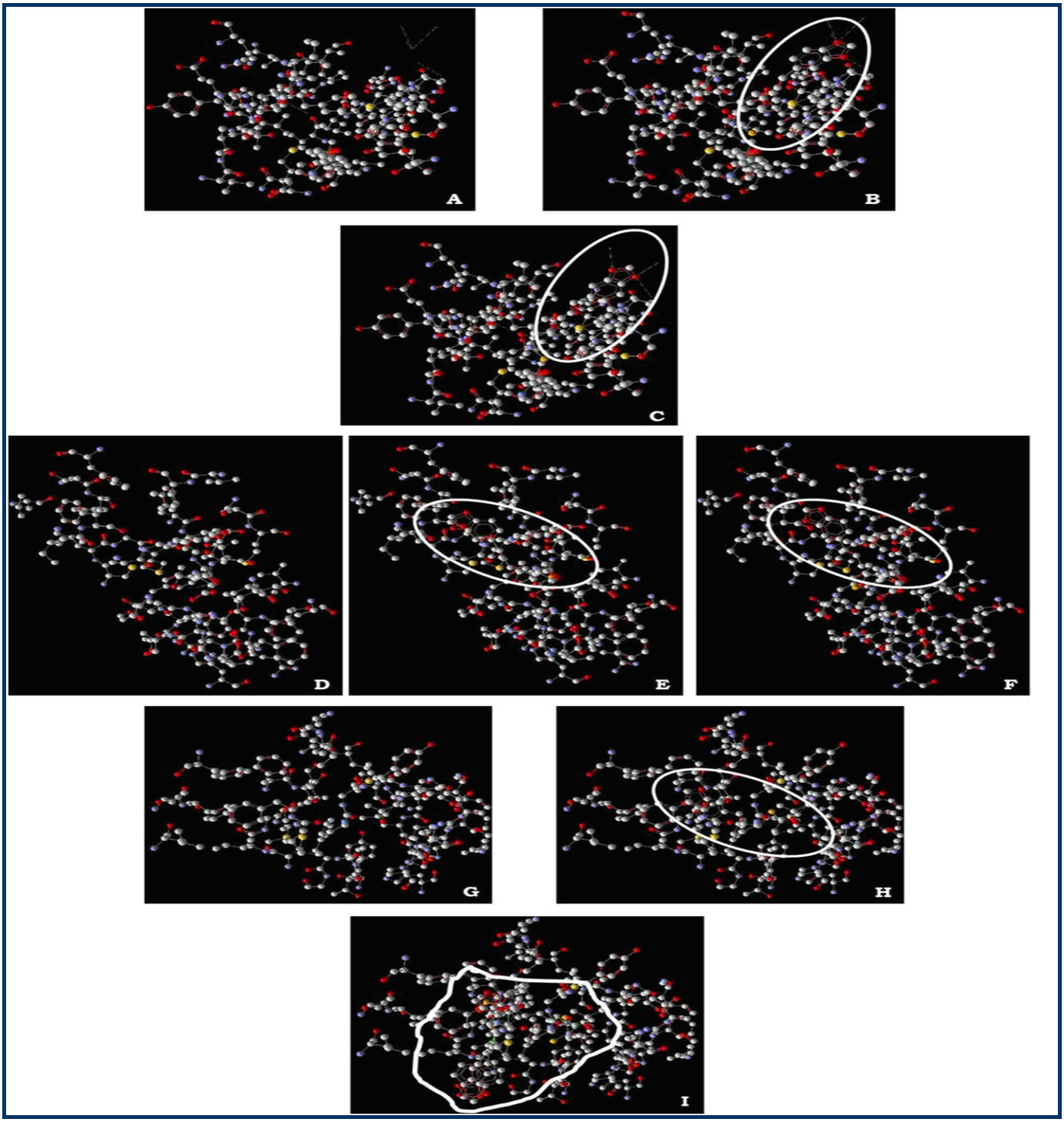

Figure 2: 2A, 2D and **2G** represent amino acids around the cavities. Figure 2B and 2C represent the ligands docked into cavities. **2E** and **2F** represent the ligands docked into **2D**. **2H** represents the Ligand docked into **2G**. **2I** represents all the five ligands docked into **2G** cavity.

Conclusion:

Many drug-drug interactions can be explained by inhibition of P-glycoprotein and/or CYP3A4. A broad variety of drugs are substrates for both P-glycoprotein and CYP3A4 and because many compounds are inhibitors of both proteins, elevated plasma concentrations of a drug by a concomitantly administered substance can be due to a dual effect on drug transport and metabolism. This was evidenced by our previous data on docking of piperine with CYP3A4, Ferritin and P-glycoprotein **[17].** In this paper the reports suggest that piperine probably influence the metabolism of iron, a substrate for CYP3A4.

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Supplementary material:

Table 1: Amino acid sequence and MolDock scores were presented in Table. From table, it is clear that Ligand with pose Unknown 1 is said to be best fit into Cytochrome p450 3A4 leading to least MolDock score and re-rank scores.

S. NO	Ligand	Cavity Volume	Amino Acid Sequence Surrounding the Cavity	MolDock score	Rerank score	RMSD	HBond
1	Piperine-Fe conjugate 1	605.696 A∧3; Proximity (A)-2.61	Ala(305,370) Phe(57,108,137,215,302,435); Arg (105,372) Thr (309); Ile (443, 301) Ser (119)	-132.788	-108.422	91.4833	-7.41695
2	Piperine-Fe conjugate 2	59.904 A∧3; Proximity(A)-2.61	Arg (162)	-127.055	-83.8605	91.487	-9.8363
3	Piperine-Fe conjugate 3	32.768 A∧3; Proximity(A)-2.61	Phe (419)	-123.969	-90.9825	89.9825	-2.5
4	Piperine-Fe conjugate 4	31.744 A∧3; Proximity(A)-2.61	Leu (133), Glu (294)	-117.297	-80.996	90.1965	-2.5
5	Piperine-Fe conjugate 5	27.648 A∧3; Proximity(A)-2.65	Leu (351.499),Lys (453), Met (450)	119.177	-98.6817	90.0128	0