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Comparative efficacy of piperine and curcumin in deltamethrin induced splenic apoptosis and altered immune functions

Anoop Kumar, Neelima Sharma *

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi -835215, Jharkhand, India

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ABSTRACT

Deltamethrin (DLM) being a potent immunotoxicant affects both humoral and cell mediated immunity. Thus, for the amelioration of its effects, two different bioactive herbal extracts piperine and curcumin are evaluated and their efficacy has been compared. The docking results demonstrated that curcumin has good binding affinity towards CD28 and CD45 receptors as compared to piperine but *in vitro* studies revealed that piperine is more effective. DLM induced apoptotic markers such as oxidative stress and caspase 3 have been attenuated more significantly by piperine as compared to curcumin. Phenotypic and cytokine changes have also been mitigated best with piperine. Thus, these findings strongly demonstrate that piperine displays the more anti-oxidative, anti-apoptotic and chemo-protective properties in the DLM induced splenic apoptosis as compared to curcumin. So, piperine can be considered the drug of choice under immunocompromised conditions.

1. Introduction

The immune system is the most sensitive target regarding toxicity of environmental toxicants. Deltamethrin is a potent immunotoxicant, causes damage to humoral immune response and cell mediated immunity. Recently, Hamid et al. [1] has demonstrated that deltamethrin induces thyroid toxicity in albino rats. We have also demonstrated DLM induced apoptosis in murine splenocytes and thymocytes, following oxidative stress and caspase dependent pathways [2,3].

Humans are exposed to DLM by various routes like inhalation, dermal, oral etc. DLM is absorbed from all these routes and finally reach systemic circulation. The spleen is the secondary immune organ which plays a major role in mounting immune responses to antigens in the bloodstream. Since DLM is a potent immunotoxicant, there is a need for studies pertaining to the identification of safe and active plant derived compounds for its attenuation.

Piperine, the main component of Piper longum Linn. and Piper nigrum Linn., is a plant alkaloid with a long history of medicinal use in Indian medicine. Piperine is the trans, trans stereoisomer of 1 piperoyl piperidine (Fig. 1a). It exhibits a wide variety of biological effects which includes antimetastatic, antithyroid, antidepressant and hepatoprotective activity [4,5]. The antiapoptotic efficacy of piperine has been reported by Choia et al. [6] against cisplatin induced apoptosis in auditory cells. In another study, it has been found that piperine protects thymocytes against DLM induced apoptosis at various concentrations (1, 10 and 50 μg/ml) [7].

Curcumin, 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione, present in the rhizome of the plant Curcuma longa, is a naturally occurring pigment and component of the spice (Fig. 1b). It possesses a wide variety of biological activities including antioxidant [8], anticarcinogenic [9] and anti inflammatory activity [10]. Regarding its antiapoptotic activity, Sood et al. [11] has shown that curcumin (10–50 μM) inhibits Shiga toxin (Stx)-induced apoptosis and necrosis in the human proximal tubule cell line (HK-2 cells). Thus, these two herbals (piperine and curcumin) may play a major role in the attenuation of DLM induced apoptosis.

Piperine and Curcumin both are potent antioxidants, but their immunomodulatory roles in the DLM induced immunotoxicity is still unexplored. Therefore, the main objective of the present study is to explore the role of piperine and curcumin in the modulation of oxidative and apoptotic effects of DLM in murine splenocytes. A docking study has been used to predict the binding affinity of piperine and curcumin towards CD28 and CD 45 receptors (B cell markers). Further, the immunomodulatory role of piperine and curcumin in DLM induced apoptosis has been compared by assessing various biochemical reflectors of cell damage such as cytotoxicity (MTT assay), oxidative stress (glutathione, reactive oxygen species), caspase 3 activity, and apoptosis along with lymphocyte phenotyping and cytokine release.

* Corresponding author. Department of Pharmaceutical Science and Technology, Birla Institute of Technology, Mesra, Ranchi -835215, Jharkhand, India. Fax: 0651 2275401.
E-mail address: nsharmabit@gmail.com (N. Sharma).

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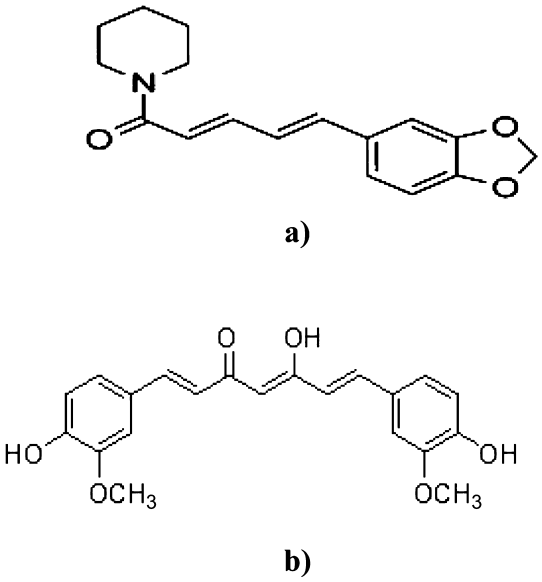

Fig. 1. a) Figure of piperine. b) Figure of curcumin.

2. Materials and methods

2.1. Chemicals

Deltamethrin (DLM), Curcumin and Piperine extract, Dulbecco's phosphate buffered saline (PBS), fetal bovine serum (FBS), 3 (4,5-dimethyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2,7-dichlorofluorescein diacetate (DCFH DA), Trichloroacetic acid (TCA), DEVD AFC substrate, Phthaldialdehyde (OPT), Dithiothreitol (DTT) and all other chemicals were purchased from Sigma–Aldrich, St. Louis, MO, USA. RPMI 1640 and Triton X 100 were purchased from Hi Media, Mumbai, India. Propidium iodide (PI) from Calbiochem (Merck KGaA, Darmstadt, Germany), FITC conjugated antiCD3 monoclonal antibody, PE conjugated antiCD19 monoclonal antibody, mouse Interleukin-2, Interleukin-4 and IFN-γ kits were purchased from e Biosciences.

2.2. Computational

All computational analyses were carried out on a Red Hat 5.0 Linux platform running on a Dell Precision workstation with Intel core 2 quad processor and 8 GB of RAM.

2.2.1. Molecular docking studies

The x-ray crystal structure of CD28 (PDB: 1YJD) and CD45 (PDB: 1YGR), with resolution 2.70°, 2.90 A^0, R-value: 0.248 (obs.), 0.255 (obs.) respectively, was obtained from the protein data bank (Research Collaboratory for Structural Bioinformatics (RCSB) (http://www.rcsb.org/pdb).

2.2.2. Protein and ligand preparation

The proteins (PDB: 1YJD &1YGR) were prepared by using the Protein Preparation Wizard. Preprocessed bond orders were assigned, hydrogens were added, metals were treated, and water molecules were deleted. Heterostate for cocrystallized ligand was generated using Epik; protonation state and optimization of H-bonding of the protein side chains were assigned using Prot Assign. Energy was minimized (Impref minimization) using RMSD 0.30 converged by OPLS 2005 force field utilities of Schrodinger's suite 8.5. The 3D structures of all ligands were drawn by using maestro, prepared by ligprep with default parameters.

2.2.3. Receptor grid generation

Receptor Grid has been generated by GLIDE module of Schrodinger with default parameters and without any constraints. The site has been specified as the centroid of the workspace ligands N acetyl D Glucosamine and ARG GLY TY.

2.2.4. Molecular docking protocol

The ligand docking was performed in GLIDE5.0 using extra precision mode with all default parameters. Best poses were chosen for energy minimization during docking, a distance dependent dielectric constant of 2.0 and the maximum number of minimization steps. The docking simulations (Ligand receptor interactions) are scored using the Xtra precision (XP) mode which is implemented in GLIDE 5.0.

Table 2
Docking of piperine and curcumin with the CD45 receptor (1YGR).

Ligand name	Amino acid residues	Interactions			No of H bonds	Glide score	e model score
		H-Bonding		Aromatic bonding			
		s.s.	b.b.	π–π stacking			
Ref	ASP705	Yes	No	No			
	LYS736	Yes	No	No	7	–8.12	–128.84
	ARG 734	Yes	No	No			
	ARG 834	Yes	Yes	No			
	ASP 796	Yes	No	No			
	GLY 833	No	Yes	No			
	VAL 832	No	Yes	No			
Piperine	ARG 834	Yes	No	No	2	–2.63	–34.97
	ARG 833	No	Yes	No			
	ASP 796	No	Yes	No			
Curcumin	ASN 735	No	Yes	No	3	–4.70	–54.44
	PYR 658	No	No	Yes			

s.s.: side chain hydrogen bonding; b.b.: backbone hydrogen bonding.

Table 1
Docking of piperine and curcumin with the CD28 receptor (1YJD).

Ligand name	Amino acid residues	Interactions				No. of H bonds	Glide score	e model score
		H-Bonding		Aromatic bonding				
		s.s.	b.b.	π–π stacking	Hydrophobic			
Ref	Glu32	Yes	Yes	No	No	1	–3.45	–22.29
Piperine	ASN53	Yes	No	No	No	1	–2.82	–20.93
Curcumin	Gln 56	Yes	No	No	No	2	–3.56	–30.45
	Tyr 51	No	No	yes	No			

s.s.: side chain hydrogen bonding; b.b.: backbone hydrogen bonding.