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# Molecular docking study of *Momordica charantia* Linn phytoconstituent with caspase 3 and implications for renoprotective actions in diabetes mellitus

**Mohan Krishna Ghanta[1], Swapna R Nayaka[1], Poojith Nuthalapati[2], AK Afzal Khan[1], Panchanathan Elango[3*], LVKS Bhaskar[4*]**

[1]Department of Pharmacology, MVJ Medical College and Research Hospital, Hoskote, Bangalore-562114, Karnataka, India
[2]PJ Biousys, Irving, Texas, USA-75038
[3]Department of Pharmacology, Bhaarath Medical College and Hospital, Agaram Main Road, Selaiyur, Chennai, Tamil Nadu, India
[4]Department of Zoology, Guru Ghasidas Vishwavidyalaya (GGU), Bilaspur-495551, Chhattisgarh, India

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**ABSTRACT**

**Introduction:** Caspase 3, an apoptosis executioner, inhibition may be beneficial for diabetes, nephropathies, neurodegenerative disease treatments and in areas of regenerative medicine. Since early traditional medicine, plant extracts comprised the major treatments of many ailments. Phytoconstituents have been a prime source for therapeutics, which are abundantly available resources. Therefore, with the interest to identify potential anti-apoptotic agents in plant extracts, D-galacturonic acid (DGA) was selected for screening anti-caspase 3 activity as it is the major constituent in *Momordica charantia* (bitter melon) and many other fruits' pectin composition.

**Objectives:** The present study aimed to evaluate activity of major phytoconstituent of *M. charantia* extract, DGA against caspase 3.

**Materials and Methods:** The chemical structure of the ligand was from obtained PubChem database, and the protein structure was procured from PDB database. Molecular docking study was performed using AutoDock version 4.2.

**Results:** This study states the interactions of DGA with GLU'124, LYS'137 and ARG'164 amino acids of caspase 3, where GLU'124, LYS'137 amino acid interactions are important for stability of caspase 3 enzyme.

**Conclusion:** The interactions between DGA and caspase 3 revealed in this study may be helpful in characterizing the medicinal property of this phytoconstituent in the bitter melon extract by future studies.

Original

*Implication for health policy/practice/research/medical education:*
*Momordica charantia* has varying actions which are proven beneficial to human health. Characterizing the phytochemicals of *M. charantia* extract and further preclinical/clinical studies may provide a potential drug candidate for renoprotection and many other ailments involving caspase 3 like neurodegenerative diseases and malignant disorders.

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## Introduction

Apoptosis is a programmed cell death mediated by many pathways including effector caspases-3, 6 and 7 which targets >600 protein molecules, and consistently linked to diabetic kidney disease (1-3). Caspase 3 and 9 were found as one of the potential factors to cause neurodegenerative diseases (4,5). Small molecule observations into their successful mediation will thus provide clues for the treatment of these pathogenesis (6). The cysteine-aspartic acid protease (caspase) family includes the caspase 3 enzyme. The sulfhydryl group of cysteine-285 (Cys-285) and the imidazole ring of histidine-237 (His-237) form the catalytic site of caspase-3. His-237 stabilizes the main aspartate residue's carbonyl group, while Cys-285 attacks to cleave the peptide bond. Through hydrogen bonding, Cys-285 and Gly-238 also help to stabilize the substrate-

***Corresponding authors**: Bhaskar VKS Lakkakula, Email: lvksbhaskar@gmail.com, and Elango Panchanathan, Email: drpelango@yahoo.com, elango.pharmacology@bmch.ac.in

enzyme complex's tetrahedral transition state. Caspase-3 prefers the peptide aspartate-glutamate-valine-aspartate-glycine (DEVDG) *in-vitro*, with dissociation emerging on the carboxyl side of the 2nd aspartic acid residue (between D and G) (7). Of 497-residue of protein, residues 124–162, are involved in enzyme inhibition (8).

There are many studies on *Momordica charantia* plant extract showing caspase 3 stimulation against some cancer experimental models (9,10). While some studies have also shown the caspase 3 inhibitory or down regulation in neurodegenerative experimental models (11-13). In context to renoprotection, *Momordica charantia* extract (MCE) has been shown to restore serum glucose levels and renal damage in Sprague-Dawley rat model, which may imply its effectiveness (14). Therefore, we planned to conduct this study to predict the role of D-galacturonic acid (DGA) as an inhibitor of caspase 3 which is one of the major phytoconstituent of *Momordica charantia*, DGA.

## Objectives

The objective of this study was to demonstrate the action of DGA against caspase 3 using *in-silico* methods.

## Materials and Methods

DGA is uronic acid, a product of oxidative reaction of D-galactose and an important compound for many metabolic reactions in human body (15). The chemical structure of the DGA was obtained from PubChem database (PubChem ID: 439215) [http://www.ncbi.nlm.nih.gov/pccompound] and the 3-D structure was extracted from Chem Draw Ultra 11.0. The structure confirmation and ligand energy minimization were conducted with PRODRG server (16). The chemical properties of ligand were explored with DataWarrior software v04.06.00. The crystal 3-D structure of protein was taken from PDB database (PDB ID: 1GFW). Binding sites of target receptors and prediction for ligand binding was explored using CASTp server.

Docking analysis was performed using AutoDock Tool v.1.5.6 and AutoDock v 4.2 programs from Scripps Research Institute (http://www.scripps.edu/mb/olson/doc/autodock) as in method described by previous study and, for PyMol molecular viewer and PoseView as well (15).

## Results

The DGA showed interactions with GLU'124, LYS'137 and ARG'164 (Figure 1). The ligand efficiency of DGA was 0.31. The inhibition constant was 1.12 µM and Van der Waal's interaction energy was -3.65 kcal/mol. The DGA interacted with caspase 3 forming hydrogen bonds at GLU'124 and LYS'137. The type of bond involved in the interaction is stated in the Table 1.

## Discussion

The DGA showed interactions with glutamate, lysine and arginine residues of caspase 3 catalytic site. The ligand efficiency of DGA was 0.31, which implies the drug-likeliness of the compound (17). The drug-likeliness properties of DGA were already reported from our previous studies (15).

MCEs obtained through water extraction and alcohol precipitation method contained polysaccharides and DGA as major constituent (13). This major constituent was taken for screening against catalytic site of caspase 3 in our study using in-silico method. DGA showed moderate binding activity with GLU 124, LYS 137 and ARG 164 amino acid residues of caspase 3 protein. Previous study on this protein structure demonstrated that any interactions or mutations between 124-162 amino acid residues of this protein may inactivate the caspase 3 protein enzyme activity (8). Another study inferred that presence of glutamic amino residues favors binding to ligands causing inhibition of caspase 3 protein activity (18). Hence DGA interactions with GLU 124 and LYS 137 may show significant role in regulation with caspase 3 activity.

In context with caspase 3 regulation with MCE, varying activity have been reported in different disease experimental models. In experimental models of cancer diseases, the caspase 3 enzyme activity is found to be down-regulated or compromised whereas the MCE

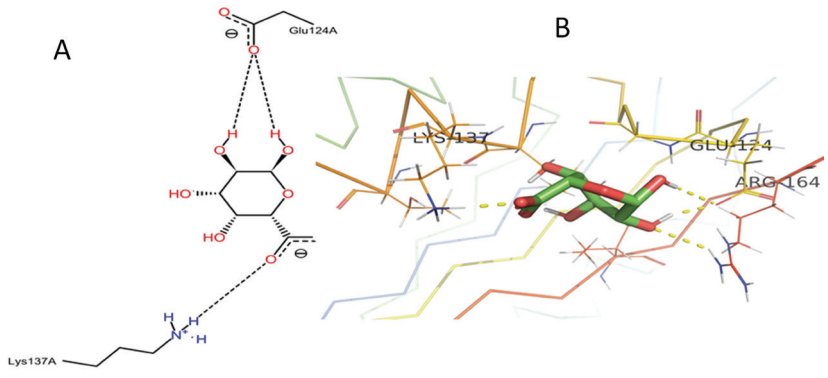


**Figure 1.** Molecular interactions of DGA with caspase 3 amino acid residues. A- 2D view, B- 3D view.

**Table 1.** Factor scores and protein-ligand complex formation

| Ligand | Protein PDB ID | Binding amino acid Residues | Binding energy (kcal / mol) | Inhibition constant uM | VDW_HB desolv_ energy (kcal/mol) | Ligand efficiency |
|---|---|---|---|---|---|---|
| D-Galacturonic acid | Caspase 3 (1GFW) | GLU`124/OE2, LYS`137/ HZ2, ARG`164/1HH2 | -4.03 | 1.12 | -3.65 | 0.31 |

GLU: glutamate; LYS: lysine; ARG: arginine; OE2, HZ2 and 1HH2 are atom names.

showed up-regulation of caspase 3 (9,10). In experimental models of neurodegenerative diseases, the caspase 3 activity was up-regulated or aggravated but MCE showed attenuation (11-13). This varying activity of MCE in previous studies and our present study results may infer that DGA exhibits mixed activity on caspase 3. This mixed function of the MCE may require further molecular experimental studies to demonstrate the MCE-caspase 3 activity. However, in this study, MCE phytoconstituent DGA showed interactions revealing inhibitory actions against caspase 3, vital for renoprotection, which has been substantiated by previous studies that caspase 3 mediates the cleavage of gasdermin E, a tumor growth suppressor, forms gasdermin E-N fragments known to change the cell death pattern from apoptosis to inflammatory necrotic events in diabetes (8,19-21).

## Conclusion

The molecular actions of DGA in relation to caspase 3 was predicted in this study which may provide insights for future studies aiming to study molecular events of MCE-caspase 3 actions and for new drug developments as well.

## Limitations of the study

This study included only DGA of MCE against anti-caspase 3 activity. Further studies are required to evaluate the role of other phytochemicals of MCE.

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## Authors' contribution

Conceptualization: MKG, LVKSB and PE. Methodology: LVKSB and MKG. Validation: LVKSB and MKG. Formal Analysis: MKG. Investigation: MKG. Resources: LVKSB, AKAK, PN and SP. Data Curation: LVKSB and MKG. Writing—Original Draft Preparation: LVKSB and MKG. Writing—Review and Editing: AKAK, PN and SP. Visualization: MKG, LVKSB and PE. Supervision: MKG, LVKSB and PE. Project Administration: LVKSB. Funding Acquisition: MKG, LVKSB and PE.

## Conflicts of interest

The authors declare that they have no competing interests.

## Ethical issues

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by authors.

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