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An Integrative Approach for *In* Silico Study on Treating Molluscum Contagiosum with Snail Slime

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Abstract: ***The dermatological disease Molluscum contagiosum (MC) presents as lesions restricted solely to the skin. The poxvirus Molluscum contagiosum virus (MCV) is responsible for this skin disease. There are four subtypes of MCV: MCV-1; MCV-2; MCV-3; and MCV-4. MCV-1 is the most commonly reported subtype found. The MCV MC159 protein belong to a family of proteins known as FLICE; FADD-like interleukin-1 β-converting enzyme-inhibitory proteins (FLIPs).MC159 is characterized by the presence of two tandem death effector domains (DEDs) that regulate/inhibit Apoptosis. Apoptosis is an effective mechanism to destroy virus-infected cells. Snail Extract contains an extraordinary combination of natural ingredients that have proven beneficial healing properties for the human skin. Snail extract is rich in natural antibiotics and hydroxyl-acids which are excellent ingredients for regeneration of new skin. Snail extract contains Allantoin, Glycolic Acid, Collagen, Vitamin E, Vitamin C, Vitamin A and Elastin-all wonderful and, very important ingredients for healthy skin. Allantoin helps reduce scars and stretch marks. This study includes inhibitory effects of Allantoin ($C_4H_6N_4O_3$), a secondary metabolite present in the Snail slime of Snail Helix Aspersa which is approved in dermatology by FDA, against MC159 protein which inhibits the Apoptosis. And further docking study was performed using Accelerys Discovery studio 4.0.Further the drug was again scanned using another chemo informatics tools to check whether it has fulfilled the condition as a drug candidate.***

Keywords: Apoptosis, MC159, MCV, Allantoin, DED

1. Introduction

Molluscum contagiosum is a disease caused by a poxvirus of the Molluscipox virus genus that produces a benign self-limited papular eruption of multiple umbilicated cutaneous tumors. Molluscum Contagiosum is a common, generally benign, viral infection of the skin. It is common in children, sexually active adults, and immunodeficient patients. It is caused by the molluscipox virus, a member of the poxviridae family. This virus differs from other poxviruses in that it causes spontaneously regressing, umbilicated tumors of the skin rather than poxlike vesicular lesions. However, when treatment is deemed appropriate, multiple local therapeutic options are available. For patients with impaired immune functions with widespread and potentially disfiguring eruptions, the usual local destructive therapies are ineffective.(Daniel and Dayna,2003).

MCV encodes proteins MC159 that inhibit apoptosis, There are four subtypes of MCV: MCV-1; MCV-2; MCV-3; and MCV-4 (Buller, *et al.*,1995). MCV-1 is the most commonly reported subtype found (Konya and Thompson, 1999). The MCV MC159 protein belong to a family of proteins known as FLICE;FADD-like interleukin-1 β-converting enzyme-inhibitory proteins (FLIPs) (Yu, *et al.* ,2008). This family of proteins was discovered during early studies of the signal transduction pathways that are responsible for the apoptosis of cells, and their discovery resulted in the identification of novel cellular FLIPs (cFLIPs) that regulate apoptosis. Here, we will review the common signaling events involved in apoptosis and then the mechanism that MC159 and its homologs use to inhibit apoptosis(table 1).(Crystall and Joanna, 2013).

Table 1: MC159 Proteins Function and Mechanism

MCV protein	*Function*	*Mechanism*	*Homologs*
MC159	Inhibits apoptosis and inhibits NF-κB	Binds FADD and IKKγ	MCV MC160, KSHV K13 and cFLIPs

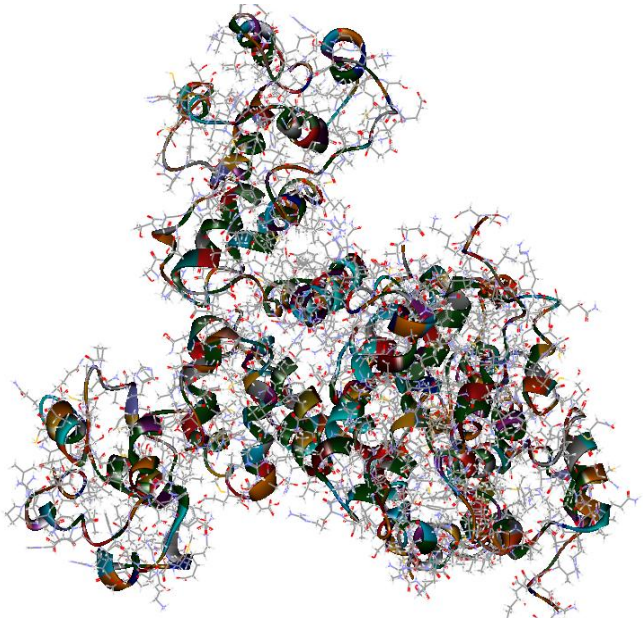

Figure 1: Protein Structure

Table 2: Protein structural information from PDB

Header	VIRAL PROTEIN/ death effector domain
Method	X-RAY DIFFRACTION
Experiment	Resolution: 3.8 Å, R-factor: 32.5%
Primary citation	Viral CASP8 and FADD-like apoptosis regulator
Organism	Molluscum contagiosum virus subtype 1

The MC159 protein (Figure 1) inhibits TNFR-1-induced apoptosis (Figure 1) The molecular mechanism of the

MC159 protein in this apoptosis pathway has been intensely studied. Garvey *et al.* show that prevention of apoptosis by the MC159 protein is correlated with MC159 binding to either FADD or procaspase-8 through an RXDL motif present in each of the MC159 protein DEDs. Moreover, both DEDs of MC159 must be present for MC159 to provide its antiapoptotic function (Garvey, *et al.*,2002).Structural studies suggest that the MC159 protein binds FADD, thus preventing its self-oligomerization and, in turn, death-inducing signaling complex assembly (Yang, *et al.*,2005; Li, *et al.*,2006).

Apoptosis is an effective mechanism to destroy virus-infected cells (Crystall and Joanna, 2013).For apoptosis, TNF–TNFR-1 interactions initiate recruitment and activation of DISC(Death-inducing signaling complex), composed of TRADD, FADD and procaspase-8. The MC159 protein binds to FADD and prevents DISC assembly, inhibiting TNF-induced apoptosis during MCV infection. Central to this process is the interaction of FADD and procaspase-8 (Muzio, *et al.*, 1996).During TNFR-1-induced apoptosis, FADD interacts with procaspase-8 via DED motifs that are present in both proteins. Next, procaspase-8 undergoes autoproteolysis, in which the N-terminal tandem DEDs are cleaved, resulting in a mature, highly active enzyme that activates the downstream procaspase-3, which in turn cleaves other proteins to initiate apoptosis.

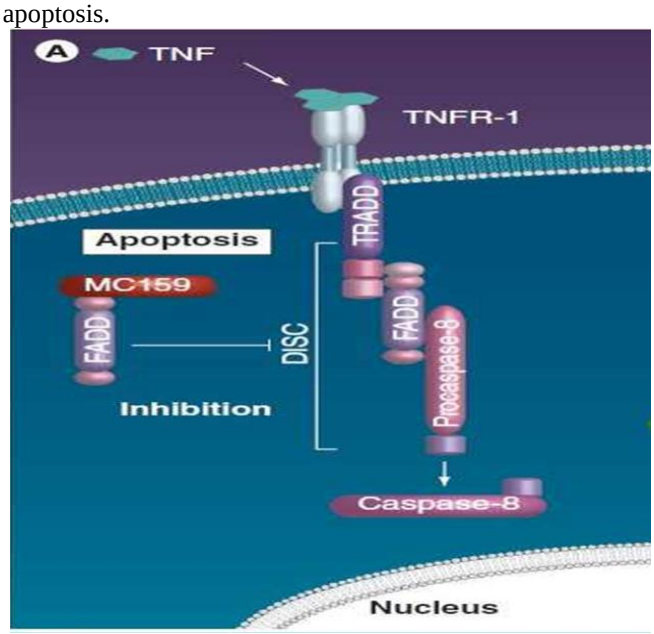

Figure 2: Mechanism of apoptosis and affected FADD with binding MC159

Snail Extract from Helix Aspersa contains an extraordinary combination of natural ingredients that have proven beneficial healing properties for the human skin. Snail extract is rich in natural antibiotics and hydroxyl-acids which are excellent ingredients for regeneration of new skin. Snail extract also contains Allantoin, Glycolic Acid, Collagen, Vitamin E, Vitamin C, Vitamin A and Elastin-all wonderful and, very important ingredients for healthy skin. Collagen and elastin improve the structure and firmness of skin. Glycolic acid helps with exfoliation. Allantoin helps reduce scars and stretch marks. With this wonderful blend of ingredients, snail extract can help improve many skin conditions such as: skin blemishes, including acne, acne scars, age spots, freckles, rosacea, wrinkles, scars,(including keloid ones),stretch marks and dull complexion.

Allantoin, a component in Comfrey, stimulates tissue repair and wound healing through cell proliferation. Allantoin has also had significant effect on cellular multiplication in degenerating and regenerating peripheral nerves.Skin protectant active ingredients. The active ingredients of the products consist of any of the following, within the concentration specified for each ingredient. Allantoin, 0.5 to 2 percent and allantoin is a pharmaceutically accepted drug.

Cosmetic Creams which are available in market with **FDA** / **AFSSAPS** approvals are Elicina, Helix IR and Labcconte and more, in which had a ratio of allantoin (0.370 mg/L) 85% dimmed comparing snail slime contents of allantoin(FDA approved) whichUsed for Dermatological treatments.

The main objective of this study is to Identify and to study the Antiviral effect of Allantoin in Snail Slime on MC159 protein of Molluscum Contagiosum through***in silico studies*** like (Absorption, Distribution, Metabolism, Excretion, toxicity) properties and docking studies of the compound present in the snail slime (Allantoin) Using Accelrys Discovery Studio 4.0.

2. Materials and Methods

Database screening for retrieval of MC159 and allantoin

Protein Data Bank (PDB)
The PDB (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The MC159protein which is reasonable for the inhibition of apoptosis in Molluscum contagiosum was retrieved from PDB database which was from the organism Molluscum contagiosum virus subtype 1with an entry name 2BBZ and studied the already validated structure and its mechanism FADD-like apoptosis regulator.

Chemspider
ChemSpider is a free chemical structure database providing fast text and structure search access to over 32 million structures from hundreds of data sources. And the three dimensional structure of the ligand Allantoin was retrieved from chemspider.

Protein simulation and validation
The retrieved structure of MC159 complex from PDBwas refined by CHARMm force field (Brooke *et al.*, 1983) in DS modeling protocol, which provides powerful mechanics and dynamics protocols for studying the energetics and motion of molecules. Accelerys CHARMM force field was used throughout the simulation studies. Constraints were applied to allow only the binding sites and ligand to be flexible during the simulation. Potential energy of the modeled proteins was analyzed before and after minimization by using Calculate Energy and Minimization protocols in DS 4.0. Parameters of minimization were smart minimizer algorithm with 200 (3000 maximum) steps, 0.01 RMS

gradient and 0.0 energy changes value and electrostatic based on spherical cut off

Active site prediction

The protein MC159 was retrieved from PDB Database for molecular docking studies. The active sites of the protein were predicted using DS 4.0, which is based on the receptor cavity method using Eraser algorithm .

Ligand Minimization and Preparation

The energy of the ligand was minimized and analyzed based on the potential energy before and after minimization. The ligand was minimized using CHARM force field. Various ligand conformations were generated based on bond energy, CHARM energy, dihedral energy, electrostatic energy, initial potential energy and initial RMS gradient values**.** Once the ligand was minimized, it has to be prepared. Preparation of the ligand was done using Prepare ligand protocol in the DS 4.0. (Brooke *et al.,* 1983)

PASS (Prediction of Activity Spectra for Substances)

Is a software product designed as a tool for evaluating the general biological potential of an organic drug-like molecule.**PASS** provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. Thus, **PASS** can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing. **Pa (probability "to be active")** estimates the chance that the studied compound is belonging to the sub-class of active compounds (resembles the structures of molecules, which are the most typical in a sub-set of "actives" in PASS training set).**Pi (probability "to be inactive")** estimates the chance that the studied compound is belonging to the sub-class of inactive compounds (resembles the structures of molecules, which are the most typical in a sub-set of "inactives" in PASS training set) .

Docking Study of proteins with Allantoin

The Molecular docking studies were carried out to investigate the binding affinities and interaction modes between the inhibitor, Allantoin and the target, MC159 complex of Molluscum contagiosum using Accelerys Discovery studio 4.0. The active sites of the proteins and the screened ligand were loaded in a new molecular window and CDOCKER protocol was run. The docked ligand-targets were analyzed carefully to identify the interactions and binding affinities. The docking scores were recorded and docking poses were saved. (Wu et al., 2003).

3. Results and Discussion

Energy Calculation and Active Site prediction :

Calculate energy protocol in DS 4.0 and the results was given in (Table 3). Based on the receptor cavity method we identified 30 active sites in the protein structure. Based on the size of the volume, selected the first active site(figure 4) for further study and the ligand Allantoin was prepared

Table 3: Calculated energy of protein and ligand at minimization (before & after)

	Forcefield CHARMm	Potential Energy (kcal/mol)	vander Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	RMS Gradient (kcal/ (mol x A)
PROTEIN	Before Minimization	2384034.3	2400922.3	-24320.432	409652.48
	After minimization	-48879.06	-5053.528	-50336.177	0.86940
LIGAND	Before Minimization	-22.135	-2.9473	-41.3803	15.8179
	After minimization	-31.6888	-2.9473	-41.3803	0.00597

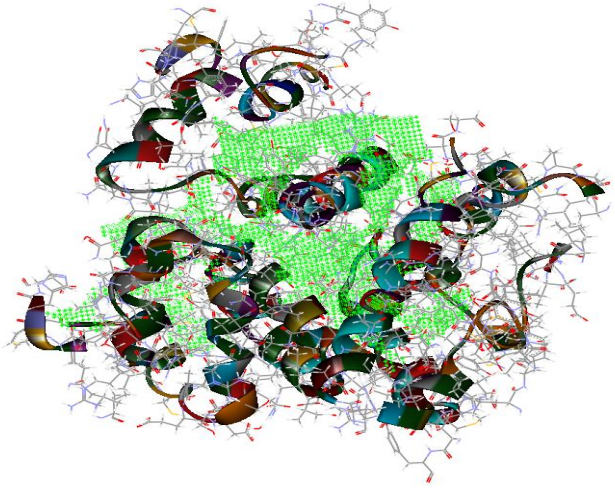

Figure 4: Active site predicted

Predicted activity with PASS server

The general biological potential of an organic drug-like molecule Allantoin was studied and given in (table 4) **PASS**result which provides simultaneous predictions of many types of biological activity based on the structure of organic compounds.

Table 4: PASS result

Pa	Pi	Activity (PASS)Prediction of Activity Spectra of Substances
0,948	0,000	Keratolytic (Wart removal)
0,778	0,002	Astringent(To shrink tissue)
0,698	0,008	Dermatologic
0,671	0,005	Antipsoriatic
0,385	0,031	Antiviral (Poxvirus)

Binding Affinity

The minimized ligand, Allantoin was docked with the active sites of the MC protein MC159 complex using Accelerys Discovery Studio 4.0. The docked ligand-targets were analyzed carefully to identify the interactions and binding affinities. Allantoin had showed interactions against MC159 proteins DED domains (Figure 5) which are reasonable for the inhibition of Apoptosis by forming 5 hydrogen bonds (table 5) with the amino acid residues at the binding site of the receptor. And a good binding energy of CDOCKER interaction -26.3734 was found (table 6).

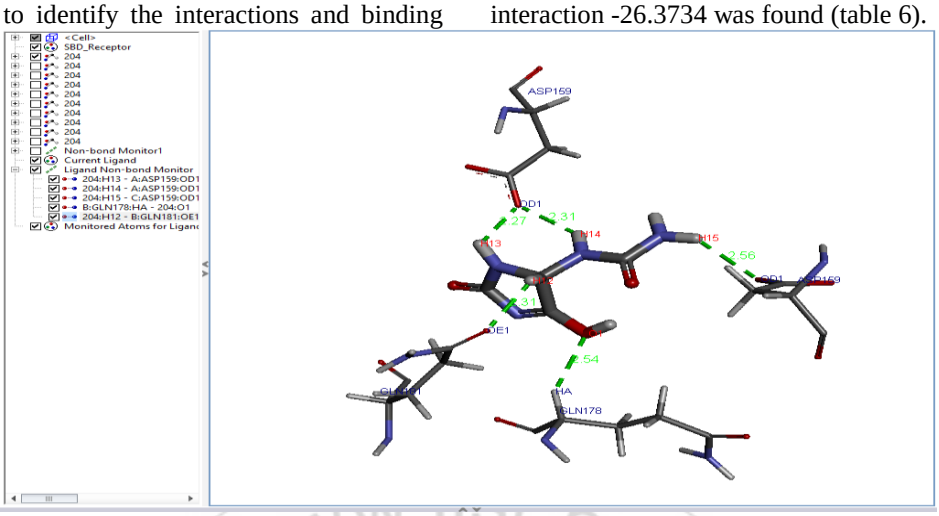

Figure 5: Visual representation of 5 hydrogen bond interaction between protein and ligand

Table 5: Interactions between ligand and protein

Ligand molecules	Bond with distance	Chain	Amino acid	Amino Acid molecule
H12	2.31	B	GLN 181	OE1
H13	2.27	A	ASP 159	OD1
H14	2.31	A	ASP 159	OD1
H15	2.56	C	ASP 159	OD1
O1	2.54	B	GLN 178	HA

Table 6: binding energy and CDOCKER energy

-Binding energy (kcal/mol)	-CDOCK interaction energy (kcal/mol)
23.9418	26.3734

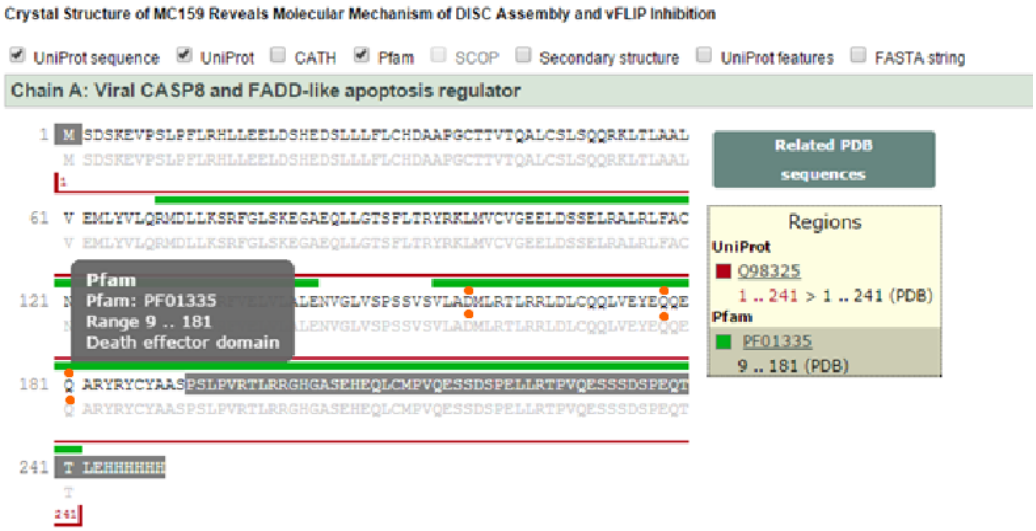

Figure 6: Three spots of interaction taken in DED domain of MC159

4. Conclusion

In the present study the activity of Allantoin, a secondary metabolite in *Snail slime* extract of Helix Aspersa against MC159protein of Molluscum contagiosum virus subtype 1virus was analyzed and also the effect of Allantoin on the MC159 was studied through Receptor-ligand binding interaction studies carried out by performing docking of the ligand Allantoin with the Protein MC159 Proteins DED domain (figure 6) which inhibit Apoptosis. From my study I found Allantoin act as an enhancer for apoptosis as it show better ligand-protein interactions and stability.

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