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Homology Modelling and Docking Analysis of *CYP41* Protein in Response to Pyrethroids in *Rhipicephalus microplus*

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ABSTRACT: Molecular docking studies are an invaluable tool to understand the interactions between proteins and ligand molecules. Interactions of pyrethroid compounds,commonly used as acaricides, with detoxifying enzyme cytochrome P450 were assessed through docking studies. The *CYP41* protein from *Rhipicephalus microplus* was modelled using Phyre[2] web portal. Protein modelling involved the construction of 3D structure of the protein and the modelled structure was further validated using SAVES sever. The modelled structure was docked with different acaricides such as flumethrin, permethrin and cypermethrin using Glide software in Schrodinger Software package. These *in silico* studies help to understand the binding affinity of protein towards ligand molecules.

KEYWORDS: *Rhipicephalus microplus*; *In silico*; docking; Acaricides; Glide; *Cytochrome P450*

I. INTRODUCTION

The prolonged and incorrect use of acaricides has led to the development of resistance in tick and mite species. Many mechanisms such as target site mutations, metabolism of acaricide before reaching its target site due to the changes in P450 monooxygenases, GSTs and esterases are involved in developing resistance [1, 2, 3, 4]. Molecular analyses of acaricide mechanisms would help in identification of genes responsible for imparting resistance and also to understand the underlying genomic level changes. *Rhipicephalus microplus*, southern cattle tick, is one of the major threats to the cattle population. It affects the global cattle and small ruminant population by transmitting babesiosis and anaplasmosis on animals, thus resulting in decreased production and restricting the traffic of livestock [5]. Synthetic pyrethroids, organophosphates, formamides etc. are the major group of acaricides used to control tick population. Among these, most commonly used acaricide class is synthetic pyrethroids. Flumethrin, permethrin and cypermethrin are the frequently used synthetic pyrethroids in the cattle field populations. These compounds are chemically modified to increase their stability and toxicity. These three acaricides are extensively used as a topical pesticide for the control of ecto-parasites such as ticks and buffalo flies on farm animals by different treatments such as dipping and spraying [6].

Molecular level analysis of resistance to the above mentioned acaricide classes were studied in many species especially in *R. microplus* [7, 8, 9, 10]. Increased levels of P450 monooxygenases and esterases are well documented in many pyrethroid resistance related studies [11,12]. *CytochromeP450* dependent monooxygenases are elementary enzyme systems involved in the metabolism of a phenomenal number of endogenous and exogenous processes [13]. It is well known that elevated activity of *Cytochrome P450* accelerates metabolism of drugs and is often responsible for drug tolerance. *In silico* analysis of the *CYP* proteins involved in resistance phenomena can help aid development of innovative resistance breaking acaricide formulations. In the current study we selected *CYP41* which is one of the identified gene in *Rhipicephalus microplus* [14].

In silico docking of these drugs in *R. microplus Cytochrome P450* protein would help to decipher their binding efficiencies and also to find out the most effective drugs among these acaricides. Molecular Docking is like a lock and

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key mechanism, where the protein of interest is referred to as lock and the ligand is referred to as key. The orientation of ligand molecule may predict the strength of association or the binding affinity to the target molecule. In this work, the 3D model of *R. microplus Cytochrome P450* protein *CYP41* was predicted and an interaction study was done with commonly used synthetic pyrethroids - flumethrin, cypermethrin and permethrin with an intention to enhance the knowledge on the interface of *CYP* protein and acaricides.

II. MATERIALS AND METHODS.

Retrieval of sequence and analysis

Cytochrome P450 *CYP41* sequence of *R.microplus* was obtained from NCBI database (www.ncbi.nlm.nih.govAccession ID : AAD54000). The protein sequence was retrieved in the FASTA format. The physiochemical properties of the selected sequence was analysed using ProtParam tool (http://web.expasy.org/protparam/), which computes various physical and chemical parameters for a given protein sequence. Secondary structure prediction of the protein was done with SOPMA server [15] (https://npsa-prabi.ibcp.fr/).

Three dimensional structure generation and validation

In this study, three dimensional structure of *CYP41* was predicted by using Phyre^2 (Protein Homology/analogY Recognition Engine V 2.0). It is one of the most reliable structure prediction softwares. The generated PDB format of the structure was viewed by using RASMOL visualisation tool. Secondary structures like alpha helix, beta sheets, random coils and extended strands were analysed using RasMol. The structure validations of the selected proteins were identified by Ramachandran plot obtained from protein preparation wizard in the Schrodinger package. The Stereochemical analysis of the generated structure was performed using SAVES server (The Structure Analysis and Verification Server version 4). SAVES server was used for the comparison for the quality of the refined structure (http://nihserver.mbi.ucla.edu/saves).

Ligands

Flumethrin, cypermethrin and permethrin, the commonly used acaricides, were selected as ligand molecules. These ligand molecule structures were obtained from Pubchem database (http://pubchem.ncbi.nlm.nih.gov/ CID:6033664, CID:40326, and CID:2912). The structure of compounds, saved in sdf format, was used further for docking studies. The properties of the selected ligands were also analysed. The ligand preparation was done by using Ligprep maestro in Schrodinger software package.

Docking Studies

Docking studies of the selected or modelled protein and the selected ligand compound was done by Schrodinger molecular modelling package. It is a computational simulation method that is used to find out the best fit and orientation of ligand molecule in to the site of the receptor. The software used for the docking study was Glide which is a ligand binding program provided by Schrodinger package. The selected protein was prepared by Protein Preparation Wizard in Schrodinger maestro. The active sites were identified by Sitemap. Glide High throughput Virtual Screening (HTVS) docking was performed to find out the best binders and the results were analysed by Glide score, Glide docking energy and Hydrogen bond.

III. RESULTS

Sequence Analysis

The retrieved FASTA sequence of the *CYP41* protein sequence was subjected to ProtParam analysis to analyse physiochemical properties. The amino acid sequence length and molecular weight of the sequence was 518 and 58860.5 respectively. The number of positively and negatively charged residues in the given sequence was found to be 63 and 62 respectively. Aliphatic index of the protein was 80.71, which is defined as the relative volume occupied by

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aliphatic side chains and is regarded as the thermal stability of globular protein. Grand Average of Hydropathy (GRAVY) of the protein was - 0.217. Atomic composition of the protein is given below.

Atomic composition:

Carbon	C	2662
Hydrogen	H	4115
Nitrogen	N	705
Oxygen	O	760
Sulfur	S	22

The SOPMA secondary structure of prediction tool predicted 42.66 % Alpha helix, 17.95% Extended strands , 10.04% beta turns and 29.34% coils in *CYP41* protein sequence.

3D Structure Generation & Validation

Three dimensional structure of *CYP41* was predicted using Phyre[2] - webportal for protein modelling . The predicted structure is shown in Fig. 1.

Fig.1. *CYP41* predicted structure in Rasmol viewer

The structure possessed 4149 atoms , 4253 bonds , 30 helices, 15 turns and 47 strands. It also contained 346 H-bonds. The predicted model of the protein has been validated by SAVES server which showed the quality of the protein. After analysis of SAVES result we observed that the overall quality of the predicted structure was 72.20. The results were analysed using Ramachandran plot, which showed that 87.2 % residues fell in the allowed region. The results are shown in Fig. 2 and Table 1.

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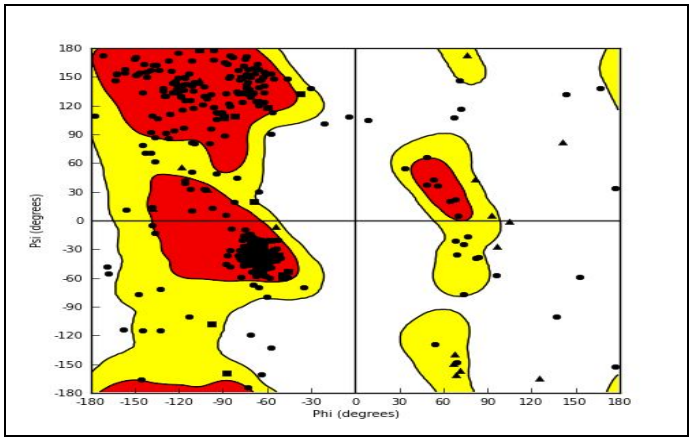

Fig.2. Ramachandran Plot of Predicted *CYP41*

Plot statistics & RASMOL Structure Details	***CYP41***
Residue in most favoured region (%)	87.2 %
Residues in additional allowed regions (%)	7.8 %
Residues in disallowed regions (%)	5 %
Number of helices	30
Number of Strands	47
Number of Turns	15

Table.1. Structure details and statistical evaluation

Docking Studies

Commonly used acaricides were selected as ligands for docking studies. The docking of the selected ligands and *CYP41* protein was done using Glide Docking Software and the results are presented in the Table. 2 and Fig. 3. Flumethrin showed -57.587 glide energy and good glide score -7.588 towards *CYP41*. Hydrogen bond interaction was seen between arginine residue (ARG460) in protein and nitrogen in ligand with a bond length of 2.14A^0 . Cypermethrin showed -41.277 glide energy and glide score -7.457. Hydrogen bond formation was observed between glycine residue (GLY388) and oxygen atom in ligand molecule. Permethrin had the least glide energy compared to the selected compounds, viz., 36.317 glide energy ,-7.639 glide score and hydrogen bond was seen between threonine residue (THR326) and oxygen atom in the ligand at a distance of 2.41 A^0 .

Table. 2 . Docking Results of the Selected ligands with *CYP41* Protein

Sl No	**Ligand**	**XP G Score**	**Glide Energy**	**H bond**			
				Number	**Protein**	**Ligand**	**H bondLength**
1	Flumethrin (6033664)	-7.588	-57.587	1	ARG 460	Nitrogen	2.14A^0
2	Cypermethrin (2912)	-7.457	-41.277	1	GLY388	Oxygen	2.06A^0
3	Permethrin (40326)	-7.639	-36.317	1	THR326	Oxygen	2.41 A^0

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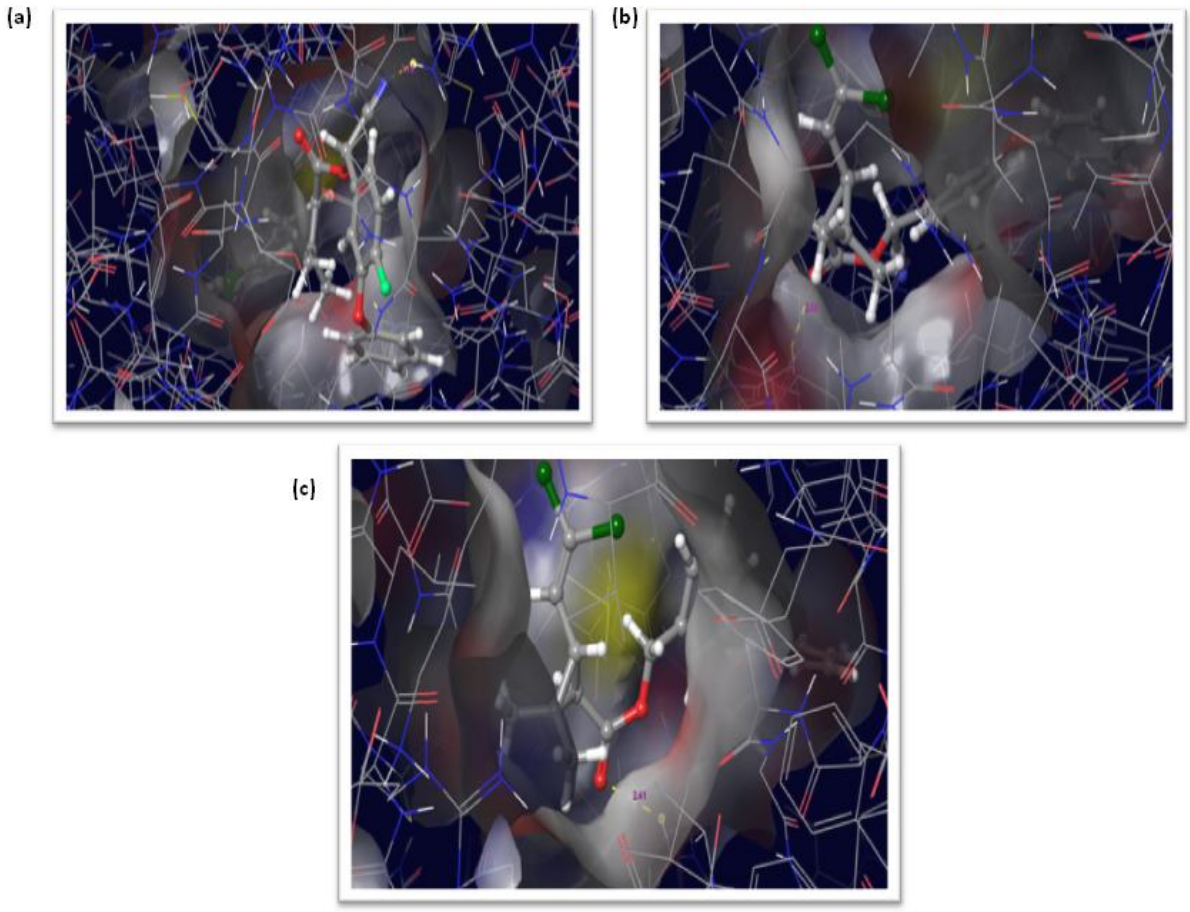

Fig.3. (a) , (b) and (c) shows *CYP41* interactions with flumethrin , cypermethrin and permethrin compounds respectively.

IV. CONCLUSION

Although synthetic pyrethroids are extensively used to control tick population, increasing cases of tick resistance against, these group of chemicals has been reported in many parts of the world recently. Therefore understanding the interaction between the drugs and their target proteins is required to control the burdens caused by these resistant tick species. One of the main genes responsible for resistance development in ticks is the *Cytochrome P450* which is involved in the detoxification mechanism. Interaction study between *CYP450* and acaricides will be useful for understanding the activity of the protein. In the present work, three-dimensional structure were predicted for *CYP41* protein and with the help of *in silico* docking study, we were able to predict the binding sites and also understand the binding activity of acaricides on *CYP41*. Among the selected acaricides, flumethrin showed highest docking score with good affinity towards the target protein molecule. The 3D structural features of the protein were useful in better understanding the activity of the protein. The predicted models and interaction studies would provide valuable insights towards the development of novel drugs.

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