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Potential honey bee *(Apis mellifera)* allergens associated with IgE-mediated allergy- An *In-silico* study

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ABSTRACT

Allergies due to honeybee venom (HBV) are reported to be the second most common form of allergy to Hymenoptera venom that occurs after being stung. Indeed, 15-20% of people test IgE positive after being stung. However, accurate data on the incidence of honey bee allergens is missing and estimated to be less than 0.001%. Beekeeping is an ancient and widely practiced activity across the Kingdom of Saudi Arabia. Still, studies on the allergenic effect of the different subspecies of honey bees are very rare in Saudi Arabia. Hence, in this study, using the *In-silico* approach, we aimed to study and evaluate the effect of allergens from honey bees in Ha'il City, Saudi Arabia on IgE-mediated allergies. A list of potential allergens from *Apis mellifera* was prepared, and the 3D structure was prepared using the SWISS-MODEL web server and the PDB database was used for retrieving the structure of the immunoglobulin E- fragment antigen-binding (IgE-Fab) region. Molecular docking (clusPro webserver) and molecular dynamics (Schrödinger) results revealed that the B2D0J5 protein from *Apis mellifera* might be the key protein associated with IgE-mediated allergic response. Overall, the identified knowledge can be used for exploring prophylactic vaccine candidates and improving the diagnosis of allergic reactions to honey bees in the Ha'il region of Saudi Arabia.

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Introduction

Bees are among the world's most common pollinators in native agricultural ecosystems (1). Honeybees belong to the family Apidae, which has more than 5,700 described species. The family Apidae (subfamily- Apinae) is comprised of a single genus, *Apis*. *Apis mellifera*- The Western honey bee is mainly found in Africa, Europe, and Western Asia region (2). *Apis mellifera* is extensively domesticated for honey and other components of biomedical importance (3). Despite the very importance of honeybees, anaphylaxis is majorly reported to be associated with honeybee venom. Allergies due to honeybee venom (HBV) are reported to be the second most common form of allergy to Hymenoptera venom that occurs after being stung (4). The prevalence of honeybee venom (HBV) allergy is highest among beekeepers and their family members, children, and farmers (5). Indeed, 20% of the world's population has IgE-specific to Hymenoptera venom, with 30% harboring IgE antibodies to bee venom. The accurate data on the incidence of honey bee allergens is missing and estimated to be less than 0.001% (5). According to a report, 15–20% of people test IgE positive after being stung with no serious symptoms (6). Adults are prone to allergic reactions as compared to other age groups (7). The type I hypersensitivity reaction is believed to be the most common mechanism mediating the Hymenoptera venom allergy (HVA) response, but the exact mechanism remains unknown (8). Interestingly, beekeeping is an ancient and widely practiced activity across the Kingdom of Saudi Arabia (9). Ha'il City is located in the Northwest part of Saudi Arabia. Ha'il City is characterized by geographically diverse terrain with a moderate climate which is suitable for beekeeping (10). Still, studies on the allergenic effect of the different subspecies of honey bees are very rare in Saudi Arabia. The allergenic proteins are believed to be involved in IgE-mediated hypersensitivity but the exact proteins and mechanism remain unknown. Computational *in-silico* studies have the advantage of screening and predicting allergenic proteins among a large set of proteins and narrowing down to the potential allergens for *in-vitro* studies. Hence, in this study, we aimed to study, evaluate,

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and predict the effect of allergens from honey bees in Ha'il City, Saudi Arabia, on IgE-mediated allergies through an *in-silico* approach.

Materials and Methods

Allergen protein sequences retrieval and modeling

A list of potential allergic proteins of *Apis mellifera* was prepared using literature (PubMed- https://pubmed.ncbi.nlm.nih.gov/) and database search (UniProt-https://www.uniprot.org/). The keyword "*Apis mellifera* Allergens" was used for searching allergenic from UniProt and literature search from Pubmed. Sequences of identified allergens were retrieved from the UniProt database in FASTA format (11). The sequences were used to predict the 3D structure of allergens using the SWISS-MODEL web server and validated using Ramachandran plot (Supplementary Figure 1). The 3D structure of the IgE-Fab region was downloaded from the PDB database (PDB ID-2VXQ) (12).

Physiochemical parameters evaluation of allergens

ProtParam server was used to predict the physicochemical properties of proteins of allergens like theoretical parameters such as isoelectric point (pI), molecular weight (MW), instability index, and grand average of hydropathicity (GRAVY) (13).

Allergenic protein and epitope prediction

The selected allergenic proteins were subjected to ALGPRED (https://webs.iiitd.edu.in/raghava/algpred2/batch.html) server to predict their allergenicity potential and identify the epitopes that might be associated with the IgE-mediated allergies (14).

Protein-protein docking studies

The IgE-Fab region consisting of heavy and light chains (PDB ID- 2VXQ) was used for screening the most likely protein involved in the interaction. For docking, the structure of IgE-Fab was prepared and refined using the Maestro package in Schrödinger Suites 2020. Missing side chains and hydrogen atoms were added, bond orders were assigned, and restrained minimization was performed using the OPLS3e force field. Ramachandran plots were used for validating the model's allergens (15). For docking clusPro webserver (https://cluspro.org/home.php) was used (16). The 3D structure of the IgE Fab region (receptor) and predicted allergens (ligands) were uploaded, and docking was performed using antibody mode with no restraints. The clusPro webserver generates four sets of models using the scoring schemes (i) balanced, (ii) electrostatic-favored, (iii) hydrophobic-favored, and (iv) van der Waals + electrostatics (16). Finally, the docked complex was visualized and analyzed using the "Protein Interaction Analysis" module in Schrödinger (17).

Molecular dynamics (MD) simulation studies

The stabilities of allergens and IgE-Fab interactions were confirmed by subjecting the docked complex to a simulation (molecular dynamics) study (18). The MD simulation was performed using Desmond (MD simulation package from Schrödinger). The docked complex was subjected to the system builder tool for solvating and neutralization (by adding counter ions). The steepest descent steps method was used for minimizing the system. Further, the full system was subjected to heating gradually from 0 to 310 K followed by the thermostat method and pressure relaxation method for 5 ns each, respectively. A total of 100 ns simulation was performed and trajectories (5000 frames) were generated at every 10 picosecond (ps). Mainly, the RMSD (Root Mean Square Deviation), RMSF (root mean square fluctuation), and Rg (radii of gyration) of the IgE-Fab-Allergen docked structure were used for the final interpretations (19).

Results

The 3-D structures of 10 potential allergens B2D0J4, B2D0J5, C9WMM5, P00630, P01501, P83563, Q5BLY4, Q5BLY5, Q5EF78, Q8MQS8 and IgE-Fab region (PDB ID-2VXQ) consisting of heavy (blue) and light (red) chains are shown in Figure 1. The molecular weight of potential allergens ranges from 2847.49 kDa to 85581.89 kDa (Table 1). The allergen P01501 has the highest hydropathicity (GRAVY) score in comparison to others. The theoretical isoelectric point for allergens ranges from 4.4 to 12.02 (Table 1). All allergens have an aliphatic index of more than 50 suggesting higher thermostability of allergens proteins.

The potential allergens were screened for their allergenic properties using a dedicated web server, i.e., "AlgPred 2.0" designed to identify the allergenic regions in the uploaded protein sequence. The server consists of large training and testing datasets (allergens (10, 075) and non-allergens (10, 075)) (14). Different score and evaluating parameters like machine learning based, MERCI-motif-emerging and with classes-identification, BLAST (Basic Local Alignment Search Tool), and hybrid approaches combing all and one and two approaches from these were used to predict the potential allergens (20, 21) . All 10 proteins from *Apis mellifera* tested for allergenic properties were predicted to be allergens by AlgPred 2.0 server (Table 2). All the predicted proteins have a hybrid score of > 0.9 (Table 2).

Further, the allergenic epitopes of predicted allergens were also identified using the IgE epitope mapping function of the AlgPred2.0 webserver. Data from the Immune Epitope Database (IEDB) (consisting of 1,584,908 peptidic epitopes) was employed to predict the allergenic epitopes from the potential allergen list (22). Out of 10, no

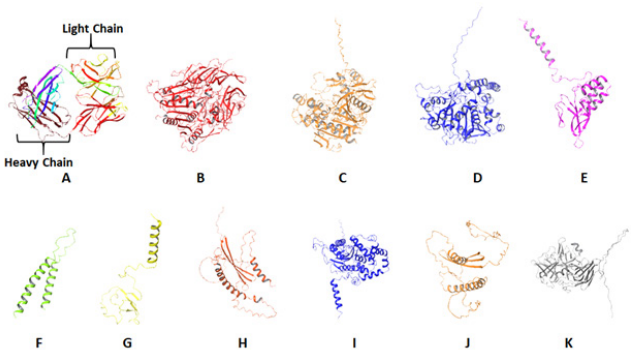

Figure 1. The 3-D structures of A) IgE Fab region (PDB ID-2VXQ), B)- B2D0J4 Allergen, C)- B2D0J5 Allergen, D)- C9WMM5 Allergen, E)- P00630 Allergen, F)- P01501 Allergen, G)- P83563 Allergen, H)- Q5BLY4 Allergen, I)- Q5BLY5 Allergen, J)- Q5EF78 Allergen, K)- Q8MQS8 Allergen.

Table 1. Physiochemical properties of Saudi Honey bee (*Apis mellifera*) allergens.

S.No	Allergens	Number of amino acids	Molecular weight	Theoretical Isoelectric point (pI)	Instability index	Aliphatic index	Grand average of hydro-pathicity (GRAVY)	Atomic composition	Extinction coefficients	Estimated half-life
1	B2D0J4	752	85581.89	5.74	35.48	79.44	-0.425	C-3841 H-5838 N-1038 O-1155 S-17	145010	1.3 hours
2	B2D0J5	536	61026.92	9.27	42.48	79.12	-0.276	C-2785 H-4231 N-731 O-775 S-21	102595	1.1 hours
3	C9WMM5	449	51561.76	6.21	32.35	85.55	-0.385	C-2370 H-3593 N-601 O-673 S-9	85845	1.1 hours
4	P00630	134	15249.25	8.07	31.04	51.64	-0.66	C-664 H-1012 N-186 O-202 S-13	23545	20 hours
5	**P01501**	26	2847.49	12.02	44.73	135	0.273	C-131 H-228 N-38 O-32 S-0	5500	30 hours
6	P83563	71	7598.11	9.7	42.72	60.28	-0.11	C-329 H-535 N-103 O-84 S-10	2115	1.1 hours
7	Q5BLY4	204	22726.99	4.4	43.54	76.86	-0.566	C-985 H-1550 N-270 O-339 S-4	26470	1.1 hours
8	Q5BLY5	373	43905.19	5.63	53.69	92.76	-0.43	C-2015 H-3018 N-503 O-579 S-9	72450	1 hours
9	Q5EF78	223	24818.55	4.51	44.12	79.96	-0.403	C-1085 H-1701 N-293 O-361 S-6	31970	30 hours
10	Q8MQS8	405	45516.57	8.64	35.98	84.69	-0.148	C-1848 H-2876 N-492 O-547 S-25	73310	0.8 hours

Table 2. ALGPRED results of allergens.

S.No.	Allergens	ML Score	MERCI Score	BLAST Score	Hybrid Score	Prediction
1	B2D0J4	0.98	0	0.5	1.48	Allergen
2	B2D0J5	0.91	0.5	-0.5	0.91	Allergen
3	C9WMM5	0.92	0.5	0.5	1.92	Allergen
4	P00630	1	0.5	0.5	2	Allergen
5	P01501	1	0.5	0.5	2	Allergen
6	P83563	1	0.5	0.5	2	Allergen
7	Q5BLY4	1	0	0.5	1.5	Allergen
8	Q5BLY5	1	0.5	0.5	2	Allergen
9	Q5EF78	1	0	0.5	1.5	Allergen
10	Q8MQS8	1	0	0.5	1.5	Allergen

Table 3. Predicted IgE epitopes of B2D0J5 allergen.

S.No.	Allergens	Epitope	Type of Epitope
1	B2D0J4	No epitope found	NA
2	B2D0J5	IDEA	Allergen
3	C9WMM5	IDEA	Allergen
		PSN	Allergen
4	P00630	CGERTEGRCLHYTVDKSK	Allergen
		CLHYTVDKSKPKVYQWFD	Allergen
		KMYFNLIDTKCYKLEHPV	Allergen
		LIDTKCYKLEHPVTGCGERTEGRCLHYTVDKSKPKVYQWFDLRKY	Allergen
		NLIDTKCYKLEHPVTGCG	Allergen
		TEGRCLHYTVDKSKPKVY	Allergen
		TISSYFVGKMYFNLIDTK	Allergen
		TKCYKLEHPVTGCGERTE	Allergen
		TNTASHTRLSCDCDDKFYDCLKNSADTISSYFVGKMYFNLIDTKCYKLE	Allergen
		TVDKSKPKVYQWFDLRKY	Allergen
		YFVGKMYFNLIDTKCYKL	Allergen
5	P01501	GIGAVLKVLTTGLPALISWIKRKRQQ	Allergen
		KVLTTGLPALISW	Allergen
6	P83563	PSN	Allergen
7	Q5BLY4	No epitope found	NA
8	Q5BLY5	IAT	Allergen
		PSN	Allergen
9	Q5EF78	No epitope found	NA
10	Q8MQS8	No epitope found	NA

epitopes were found in 3 allergens. The highest allergenic epitopes were found in P00630 (11 epitopes) allergen (Table 3).

The IgE-mediated allergic response of predicted allergies was tested using protein-protein docking studies. The docking result revealed that B2D0J5 allergen has the highest docking score (-394.5) (Figure 2.) followed by Q5EF78 (-374.8) and Q5BLY5 (-370.5) (Table 4) and might be involved in the interaction with the IgE-Fab region.

The interaction of the best-docked complex (complex having the highest docking score), i.e. B2D0J5 allergen & IgE-Fab, was studied further and the major amino acids residues involved in the interaction are shown in Supplementary Table 1. Van der Waals forces, hydrogen bonds, and surface complementarity were found to be involved in mediating the interaction between B2D0J5 allergen and IgE-Fab (Supplementary Table 1).

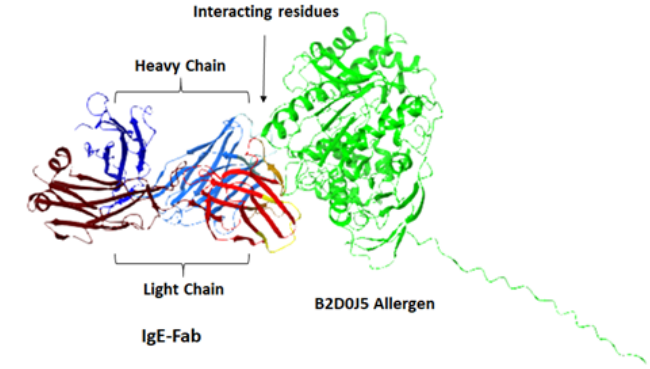

Figure 2. Docked complex of B2D0J5 allergen & IgE-Fab.

The stability of the interaction between B2D0J5 allergen & IgE-Fab was confirmed by the molecular dynamics

simulation study. The IgE-Fab- B2D0J5 (Allergen) complex RMSD was found stable i.e. 3.5 ± 1 during the overall duration of the simulation (100 ns). The major fluctuations were observed between 56 to 58 ns and 75 to 78 ns (Figure 3). Similarly, protein RMSF was observed to be stable i.e. 1.6 ± 1 throughout the simulation duration of 100 ns (Figure 3).

Discussion

In literature, very few proteins like phospholipase A2 (cytotoxic effect), hyaluronidase, acid phosphatase, and melittin (hemolytic properties) are reported to be associated with allergy (23). However, their involvement in IgE-mediated allergy is not very well established to date. Very little is known about their biological function. Venom dipeptidyl peptidase 4 (B2D0J4) is reported to process promelittin which can modulate the activity (chemotactic) of immune cells (24). Phospholipase A2 (P00630) is known to be associated with the hydrolysis of 2-acyl groups in 3-sn-phosphoglycerides in a calcium-dependent manner. However, involvement in the allergenic-associated pathway is not known (25). Melittin (P01501) is known to have strong antimicrobial and hemolytic activity. It can bind to the negative charge membrane and can form pore thus causing the lysis of the cells (26). Allergen Api m 6.03 (P83563) is reported as a protease inhibitor (27).

Previously, several studies have reported the utility of using computational tools for identifying allergens and validated their results using in-vivo studies. For example, the antigenic determinants responsible for IgE binding from *Sorghum bicolor* were identified using *in silico* modeling, docking, and simulation studies. Further, they validated their result in a mice model and shown PF3 peptide (predicted allergenic peptide) showed increased levels of IL5, IL12, TNF-alpha, and GMCSF in comparison to others (28). A similar approach was also reported to predict allergens from *Aedes aegypti* associated with allergic respiratory diseases (29). Recently, a chemometric-based machine learning approach was reported to predict the allergenicity of plant proteins as a way to control the problem of food allergies (30). Despite the several reported successful prediction story of allergenic proteins, there is some limitation associated (selection of scoring function, issue in considering the flexibility of ligand and receptor molecules, solvation effect) with computational docking and simulations algorithm and it is strongly recommended to validate the prediction using in vitro and/or in vivo assays (31). To date, no *In-silico* studies exploring IgE-mediated honey bee allergies have been reported so far in the world and the Ha'il region of Saudi Arabia (32). There have already been some studies related to the molecular genetics of honey bees (33-35). The potential allergens predicted using our immunoinformatic approach can be used to design potential prophylactic peptide vaccine candidates for reducing the allergic response associated with honey bee venom in the Ha'il region of Saudi Arabia and regions/countries (China, Turkey, Canada, United States and India) prone to honey bee attack and farming. Also, these identified proteins have additional utility as a screening marker for developing a diagnostic panel for detecting honey bee-associated allergy in patients presented with a severe allergic response (36). On the other hand, the identification of molecular mechanisms eliciting the allergic response can be further exploited to design small molecule/natural inhibitors for controlling IgE-mediated inflammation.

The role of honey bee allergens in mediating IgE is not explored much, this is the first study where the In-silico approach has been applied for the same. Overall, this study has predicted the allergenicity potential of 10 potential allergens and identified the epitopes present in honey bees found natively in Saudi Arabia, specifically the Ha'il region, and might be involved in the interaction with IgE. Our molecular docking and MD simulation results revealed that the B2D0J5 protein from *Apis mellifera* might be the key protein associated with IgE-mediated allergic response. These protein allergens can cause allergic reactions in humans. However, in-vitro studies are required for validation. Further, the identified knowledge can be used for exploring prophylactic vaccine candidates and improving the diagnosis of allergic reactions to honey bees in the Ha'il region of Saudi Arabia. This study may specifically help the people living in the Ha'il region, as it is very important for them and everybody else to be aware of the signs and symptoms of an allergic reaction and to seek medical attention immediately if they experience an allergic reaction due to honey bees.

Table 4. Docking scores of Saudi Honey bee (*Apis mellifera*) screened against IgE-Fab antibody (PDB ID-2VXQ).

S.No.	Allergens Uniprot ID	Docking score (kcal/mol)
1	B2D0J4	-359.3
2	B2D0J5	-394.5
3	C9WMM5	-368.2
4	P00630	-312.1
5	P01501	-291.5
6	P83563	-312.7
7	Q5BLY4	-366.2
8	Q5BLY5	-370.5
9	Q5EF78	-374.8
10	Q8MQS8	-337.4

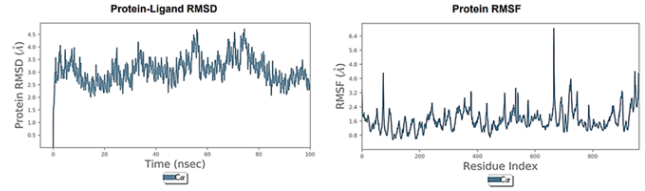

Figure 2. MD simulation studies of B2D0J5 & IgE-Fab docked complex A) RMSD plot B) RMSF plot.

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Conflicts of interest

"Authors declare no conflicts of interest."

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