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Validation of *Solanumnigrum* Proteins Asscorpion Antitoxins

Faten Dhawi[1]* and Sumayah I. Alsanie[2]

[1]Biotechnology Department, College of Agricultural and Food Sciences, King Faisal University, Saudi Arabia
[2]Biology Department, College of Science, Imam Abdulrahman Bin Faisal University, Saudi Arabia

Abstract

Therapeutic plants such as Solanumnigrum have been utilized as treatment of venomous bites. Combining these plants with antiserum is hypothesized to result in a better outcome more than utilizing of either treatment alone. Hence, this study points to validate the utilize of Solanumnigrum as an antivenom by extricating and recognizing the proteins of this plant.The sequences of 358 Solanumnigrum proteins were submitted to PSI-Blast to search for homologous snake proteins. The resulting 80scorpion proteins were subjected to multiple sequence alignments with the 358 Solanumnigrum proteins. A phylogenetic tree analysis resulted in 79 nodes in 26 clades, three of which contained scorpion proteins clustered with similar Solanumnigrum proteins. Homology modeling and structural comparison showed that three clustered scorpion proteins (2M01, 4AEI and 1J5J)were similar to proteins from *Solanumnigrum*. These scorpion proteins2M01, 1J5J and 4AEI shared at least 5% similarity to *Solanumnigrum* Q31952, P93847 and P27322 respectively.Themethod used in the current study validates the use of several Solanumnigrum proteins as agents to reduce the toxicity of scorpions' bites.

Keywords: Solanumnigrum, proteins, antivenom, scorpion, structural comparison, homology modeling

Introduction

The toxins that cause neurologic dysfunction produced by venomous snakes, arthropods such as scorpions, spiders and bacterial poisons such as botulinum toxin. These toxins commonly act on neuromuscular transmission but in certain situations the toxins interfere with some neurotransmitters and can cause respiratory paralysis in severe cases. While some toxins have proved to be valuable pharmaceutical agents, others are widely exploited to study neuromuscular physiology and pathology (Kularatne and Senanayake, 2014). The use of medicinal plants to local treatment of venomous bites known to reduce the severity of bites when combined with protein antinodes. The extract of two plant species Bouvardiaternifolia, Aristolochiaelegans strongly inhibited the lethality of Centruroideslimpiduslimpidus scorpion venom (Jiménez-Ferrer et al., 2005).

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Venomous invertebrates' bites such as scorpion from might be associated with the acute onset of medical emergencies and in severe cases they cause around 137,880 deaths and numerous permanent disabilities worldwide (Williams et al., 2019). The proteins in the venom can be used for drug discovery and the design of possible templates (Munawar et al., 2018). The toxicity caused by the bite is treated by neutralization, which can be accomplished by using its endogenous proteins or remedies composed of certain medicinal plants. For many years, valuable medicinal plants have been used as immediate treatment of snake or scorpion bites (Panfoli et al., 2010). For instance, at a low dose (0.17 mg) of Mimosa pudica root significantly reduces the lethality caused by the venom of some snakes (Meenatchisundaram and Michael, 2009).

Plants in the Solanaceae family were found to be the most frequently used for snakebite remedies (Shah et al., 2018). Solanine (Steenkamp et al., 2002), a glycoalkaloid, is a bioactive compound extracted from the berries of Solanumnigrum, which is used as a food as well as a medicinal plant (Noumedem et al., 2013). Solanine is found in all parts of the plants in the Solanaceae family, including Solanummelongena, Solanumtuberosum, and Solanumlycopersicum. These plants are replete with secondary metabolites that have practical value; for example, steroidal alkaloids from Solanumcampaniforme neutralize the myotoxicity and skin necrosis induced by the crude venom of Bothropspauloensis (Torres et al., 2011; 2013). Similarly, a glycoprotein from Solanumnigrum was observed to exert anti-inflammatory activity that decreased the number of viable HT-29 cells (human colon cancer cell line) (Lim, 2005) and MCF-7 cells (breast cancer cell line) (Heo and Lim, 2005) in a dose-dependent manner. This protein is also considered a natural anticancer agent (Lim, 2005).Solanumnigrumhas the ability to neutralize venom enzymes (Singh et al., 2017) and induce anti-inflammatory activity (Lim, 2005; Heo and Lim, 2005). Some tribes applied the leaf juice of Solanumnigrum locally on wounds caused by snakebites (Jain et al., 2011), or they mixed a fruit paste of Solanumnigrum with the leaves of Heteropogoncontortus (Kadel and Jain, 2008). Hypothetically, combining the compounds isolated from these plants with antiserum may produce better outcomes of venom toxin neutralization compared with either treatment alone. In a previous study, the computational analysis showed that two of Solanumnigrum proteins shared similarity of 18.75% with venomous snakes Najaatra. The potency of antivenom antibodies can be increased with the use of highly concentrated plant protein extracts (Dhawi, 2019). On another study, the assessment of the antioxidant effect of two leave extracts of *Solanumnigrum* L. showed inhibition of glutamate uptake, glutamate excitotoxicity and restore the oxidative status in in vitro primary cultures of rat astroglial cells exposed to glutamate (Campisi et al., 2019).

Methods

Plant material

Samples of *Solanumnigrum* were collected from Al Jubail (27.123450, 49.537920), Saudi Arabia were treated as described in Dhawi, 2019 previous study. The collected samples were placed on tissue paper and then pressed between hard boards, with the upper board applied evenly to weigh it down and flatten the samples. The samples were dried for 21 days. The dried plant samples to

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be processed for protein extraction were ground to powder. There were three replicates. The plant powder was dissolved in 50 mM ammonium bicarbonate solution. Proteins were precipitated with ice-cold acetone, and the protein pellets were then resuspended in 50 mM ammonium bicarbonate. After reduction by 10 mMDL-dithiothreitol (DTT) at 56 °C for 1 hour and alkylation by 20 mMiodoacetamide (IAA) at room temperature in the dark for 1 hour, the suspension was centrifuged at 12,000× *g* at 4 °C for 10 min. The proteins were washed once with 50 mM ammonium bicarbonate. Then, 100 μL of 50 mM ammonium bicarbonate and free trypsin were added to the protein solution at a ratio of 1:50 and incubated at 37 °C overnight. The samples were centrifuged at 12,000× *g* at 4 °C for 10 min, followed by the addition of 100 μL of 50 mM ammonium bicarbonate to the samples with two cycles of centrifugation. Finally, the samples were lyophilized, and the peptides were extracted to near dryness. The peptides were resuspended in 2–20 μL of 0.1% formic acid before LC–MS/MS analysis.

Data Analysis

The raw MS file protein sequences were analyzed and searched against the Solanaceae protein database according to the sample species. MaxQuant (1.5.6) software was used for quantitative proteomics MS data analyses. The parameters were set as follows: the protein modifications were carbamidomethylation (C) (fixed), oxidation (M) (variable); the enzyme specificity was set to trypsin; the maximum missed cleavages were set to 2; the precursor ion mass tolerance was set to 10 ppm; and the MS/MS tolerance was 0.6 Da. Only confidently identified peptides were chosen for the downstream protein identification analysis.

Phylogenetic Tree Analysis

The method used in phylogenetic tree analysis according to Dhawi, study in 2019. The sequences of 358 *Solanumnigrum*proteins were used to search for homologous scorpion proteins using PSI-Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) (Altschul et al., 1997), which resulted in 101 snake protein sequences. These sequences were filtered using PROMALS3D (PROfile Multiple Alignment with predicted Local Structures and 3D constraints) by aligning multiple protein sequences. The balance alignment using the two-stage alignment strategy described by Pei et al. (2003) reduced the number of protein sequences from 459 to 80 (Pei et al., 2008). The alignment parameters were as follows: identity threshold = 0.6; weight for constraints derived from sequences = 1; weight for constraints derived from homologs with structures = 1.5; weight for constraints derived from input structures = 1.5; profile–profile comparison; weight for amino acid scores = 0.8; and weight for predicted secondary structure scores = 0.2. The parameters for deriving sequence profiles from PSI-BLAST searches were set as follows: PSI-BLAST iteration number = 3; PSI-BLAST e-value inclusion threshold = 0.001; identity cutoff below which distant homologs are removed = 0.25; and the maximum number of homologs kept for PSI-BLAST alignment = 300. The parameters for detecting and using homologs with 3D structures (homolog3d) were as follows: PSI-BLAST e-value cutoff against structural database = 0.001, and the identity cutoff below which 3D structures are not used = 0.2. The multiple sequence alignment was performed using MAFFT (Katoh and Standley, 2013). The resulting phylogenetic tree was visualized and edited using iTOL (https://itol.embl.de/itol.cgi) (Letunic and Bork, 2019).

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Protein Homology Modeling and Similarity Evaluation

The sequence structures of *Solanumnigrum* and scorpion proteins found in the same clade were extracted from the Protein Data Bank (PDB) using Easy Modeller (Kuntal et al., 2010) and the Python script Homology-modeling_preprocessing.py. All the PDB files were visualized in UCSF Chimera (Pettersen et al., 2004). *Solanumnigrum* and scorpion proteins clustered in the same clades were aligned to assess their similarity.

Results

Three scorpion proteins were identified as similar to *Solanumnigrum* proteins were: 2M01, and (https://www.rcsb.org/structure/2M01 (Chen et al., 2013) , 4AEI https://www.rcsb.org/structure/4AEI (Fabrichny et al., 2012) 1J5J https://www.rcsb.org/structure/1J5J) (Korolkova et al., 2002).

The phylogenetic of tree analysis for 80 scorpion proteins showed that *Solanumnigrum* proteins clustered with scorpion proteins in three clades (Fig. 1). These cluster were: Cluster 1: 2M01 A Chain A with *Solanumnigrum* protein Q31952 (Fig. 2), Cluster 2: 1J5J A Chain A BeKm-1*Solanumnigrum* protein P93847 (Fig.3) andCluster 3: 4AEI L Chain L Fab with *Solanumnigrum* proteins P27322 (Fig. 4).

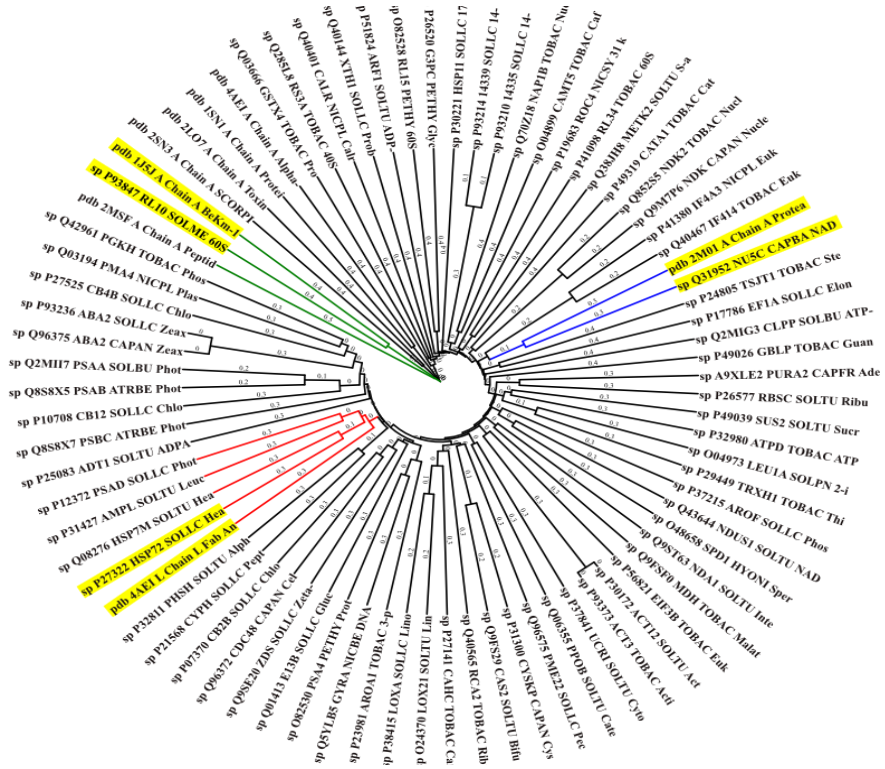

Figure1: The resulting unrooted phylogenetic tree consisted of 79 nodes, and *Solanumnigrum* protein sequences were clustered in three clades with three similar scorpion protein sequences

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(2M01, 4AEI and 1J5J). The highlighted sequence labels represent protein structures with the highest similarity.

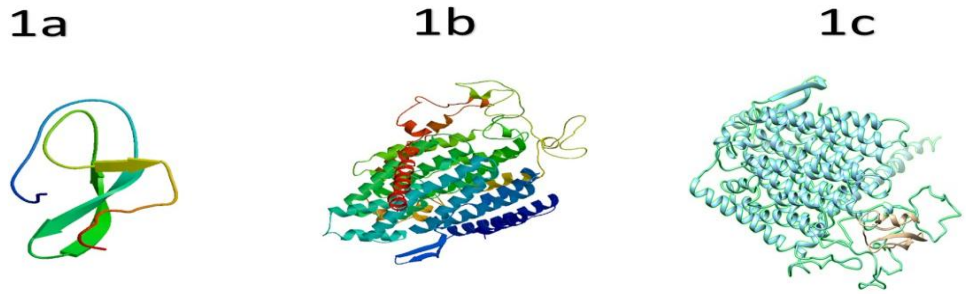

Figure2: a1)2M01 A Chain A , b1)Q31952 and c)the resulted proteins comparison between 1a and 1b with similarity of 5.08%

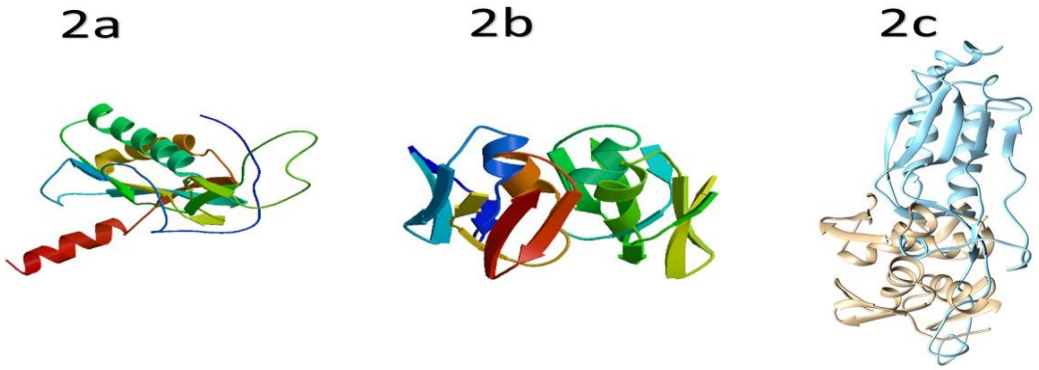

Figure3: 2a)1J5J A Chain A BeKm-12b)P93847 and 2c) the resulted proteins comparison between 1a and 2b similarity of 5.56%

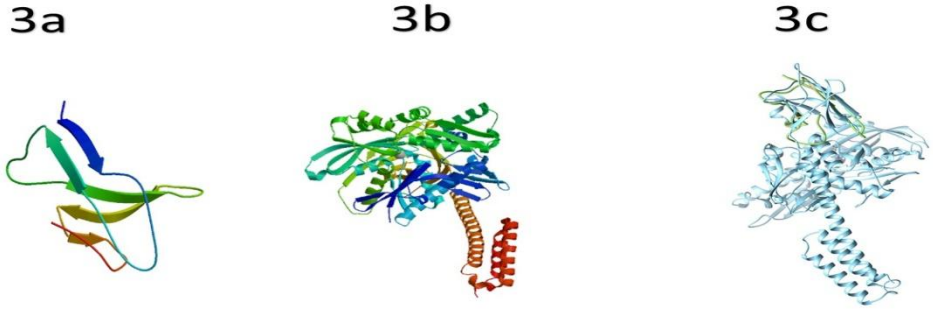

Figure 4: 3a) 4AEI L Chain L Fab, 3b) P27322 and 3c) the resulted proteins comparison between 3a and 3b similarity of 5.71%

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Discussion

The approach to neutralizing snake venom toxicity by applying serum antivenom was developed within the last century. Snakebites are treated by medicinal plants alone or in combination with processed edible items (Bhattacharjee and Bhattacharyya, 2013). Medicinal plants are vital sources of bioactive compounds that are useful for treating snakebites by boosting the effects of conventional serum therapy and reducing side effects (Singh et al., 2017). In the current study, *Solanumnigrum* proteins were clustered with the scorpion proteins 2M01, and (https://www.rcsb.org/structure/2M01 (Chen et al., 2013) , 4AEI https://www.rcsb.org/structure/4AEI (Fabrichny et al., 2012) 1J5J https://www.rcsb.org/structure/1J5J) (Korolkova et al., 2002).

2M01 A Chain A long-chain toxins a Kunitz-type fold was found in scorpion venom glands of species *Lychasmucronatus* (Chen et al., 2013) was5.08% similar to*Solanumnigrum* protein Q31952 (NAD(P)H-quinoneoxidoreductase subunit 5, chloroplastic). In addition, 1J5J A Chain A BeKm-1scorpion toxin BeKm-1 is unique among a variety of known short scorpion toxins affecting potassium channels in its selective action on ether-a-go-go-related gene (ERG)-type channels. *Mesobuthuseupeus* (Korolkova et al., 2002) was 5.56% similar to*Solanumnigrum* protein P93847(60S ribosomal protein L10).4AEI L Chain L Fab which found in scorpion of Androctonusaustralis hector (Aah) species that produce one of the most lethal venoms for humans (Fabrichny et al., 2012) was5.71% similar toSolanumnigrum protein P27322 (Heat shock cognate 70 kDa protein 2). All these results of the current study and previous study by Dhawi, 2019, showed that *Solanumnigrum*proteins have the possibility to function as co-neutralizing agents and reduce the toxicity of venoms.

Conclusion

The main conclusion of this study is that *Solanumnigrum* proteins have potential use as co-neutralizing agents to reduce the toxicity of certain scorpion venoms, such as *Lychasmucronatus, Mesobuthuseupeus*and*Androctonusaustralis.* The potency of antivenom antibodies can be increased with the use of highly concentrated plant protein extracts such as*Solanumnigrum*. Therefore, this study serves as a validation report for *Solanumnigrum*as a plant-based antivenom.

Competing Interests

The authors declares no competing financial and non-financial interests.

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