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Research Article

Insilico Targeting Biosynthetic Pathway of Aflatoxin Synthesis Using the Secondary Metabolites of *Azadirachta indica*

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ABSTRACT
Neem tree was botanically known as *Azadirachta indica*, the most traditionally used plants for many treatments in India. This tree is either called as the goddess of medicine because each part of the plant was medicinal value and contains active ingredients, thus commercially exploitable many siddhars on earlier days used neem for treating many unknown diseases. In this current study neem secondary metabolites are targeted in the biosynteic pathway of aflatoxin. The aflatoxin, which is also a mycotoxin inhibited by many synthetic drugs, but due to some side effects neem compounds has been used to inhibit the production of aflatoxin in fungus. Hence, by performing molecular docking studies and results that are obtained from docking shows that the interaction with neem compounds among 15 compounds, only Astraglin shows the better interaction with the target than the standard drugs.
Key words: *Azadirachta indica*, medicinal, secondary metabolites, aflatoxin, Astraglin.

INTRODUCTION

In all over the world herbal medicines have been the main source of health care. Herbal medicines were made from active ingredients isolated from leaf, stem, bark or other parts of the plant bodies. These kinds of plants have been catering as a rich source of safe medicines. Still today about 80% of population in worldwide depend on herbal medicines[1,2]. Either it is taken directly from one plant, sometimes a combination of plants like triphala[3]. Other cases Medicines containing plant materials combined with chemically defined, active substances, including chemically defined isolated constituents of plants are not considered herbal medicines. The current market potential of herbal medicine is estimated about $ 80-250 billion in Europe and USA, whereas in Indian herbal drug market size is about $1 billion and the export of plant based crude drug is around $100 million[1,4,5]. The plant chemicals were named as secondary metabolites and it is largely used for treating various diseases. These secondary metabolites can be classified into several groups according to their chemical classes, such alkaloids, terpenoids, etc., based on the functional pharmacophores. These kind of secondary metabolites is derived biosynthetically from plant primary metabolites as carbohydrates, amino acids, and lipids are not directly involved in the growth, development, or reproduction of plants[6-9].

Neem tree was botanically known as *Azadirachta indica*, the most traditionally used plants for many treatments in India. This tree is either called as the goddess of medicine because each part of the plant was medicinal value and contains active ingredients, thus commercially exploitable many siddhars on earlier days used neem for treating many unknown diseases[10]. Recently, over last five decades, structural chemistry of neem compounds achieved a great progress in biological activity and medicinal applications[11, 12]. The recent study on neem reports that more than ten compounds have been isolated from neem tree of various parts, structural diversity of compounds have been published by many research papers[9,12].

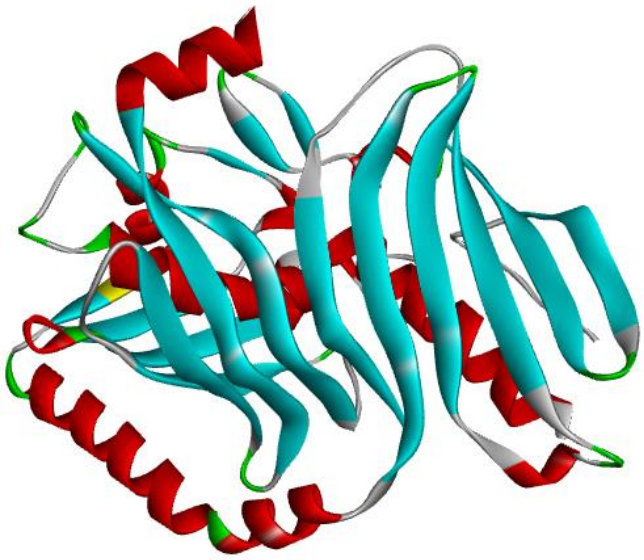

Fig 1: The secondary structure of the enzyme

Aflatoxins, are the most carcinogenic and toxic compounds and naturally occurring mycotoxins, a group

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of polyketide derived furanocoumarins[13].Nearly 25 gene cluster was identified in the biosynthesis pathway of aflatoxin,but 19 have been assigned with functions still

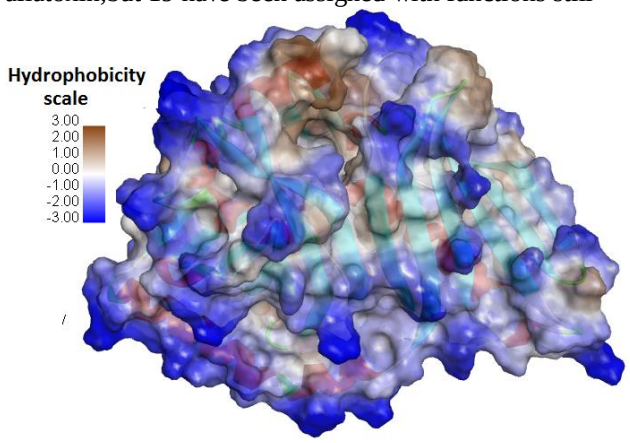

Fig 2: Polyketide synthase A with different volume of cavities based on hydrophobicity

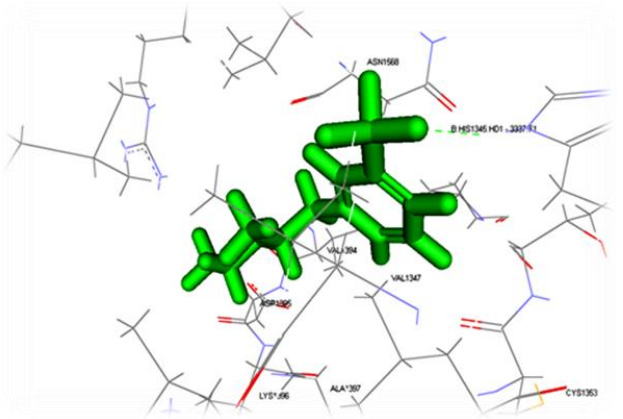

Fig 3: Standard drug docked to the active site amino acid of an enzyme

function of 6 genes were not known[14-16].Some of the researchers like Watanabe et al stated the importance of polyketide synthase in aflatoxin biosynthesis[17]. The pksA gene is weakly homologous to a PKS-encoding gene and involved in spore pigmentation was found earlier in *A. nidulans* organism, PKS gene was isolated by Feng and Leonard and named as *pksL1*, from *A. parasiticus*[17-19]. Disruption of the *pksL1* gene produced neither aflatoxins nor any aflatoxin intermediates[19]. The secondary structure of the enzyme is shown below in fig 1. In this current study neem secondary metabolites are targeted in the biosynteic pathway of aflatoxin.

MATERIALS AND METHODS

Retrieval of drug target from PDB database

The drug target protein Aflatoxin biosynthesis polyketide synthase taken from organism *Aspergillus parasiticus* with palmitic acid of alpha numeric identification 3HRQ and its x-ray crystallographic structure with 1.80 Å resolution with UniProtKB unique identification number is Q12053 with amino acid sequence of 357 was retrieved from PDB database. (http://www.rcsb.org/pdb/home/home.do). This enzyme belongs to synthases with EC number of 2.3.1.221.

Table 1: Secondary metabolites of the neem

S.No	Name of the compounds	PubChem CID
1	Astragalin	5282102
2	Azadirachtin	5281303
3	Azadiradione	363978
4	Azadirone	185587
5	Catechin	9064
6	Epicatechin	72276
7	Gallicacid	370
8	Gedunine	12004512
9	Kaempherol	5280863
10	Mahmoodin	126566
11	Morgolone	189728
12	Morgolonone	189726
13	Nimbin	108058
14	Nimbolide	100017
15	Salannin	6437066

Preparation of protein and active site prediction

The water and ligand that bound to the protein polyketide synthase was removed initially force field protocol, CHARMm was applied to remove the bad clashes and non-bonded interactions followed by the respective drug target protein was saved in the current mode of the protein data bank in discovery studio.Followed by defining the active site of the protein, which was automatically predicted using flood filling algorithm available through acclerys discovery studio 2.1v

Table 2: Docking Results of 3HRQ with Neem Compounds

S no	Compound	Poses	Dock scores in kcal/Mol
1	Azadiradione	10	40.50
2	Catechin	10	36.60
3	Nimbin	6	41.62
4	Salanin	5	36.74
5	Astraglin	7	42.93
6	Standard-Fenufluramine	10	32.59

Molecular docking

The concept of structure based drug designing was applied since the ligand and drug target protein was known, a series of natural metabolites from neem was docked with polyketide synthase to predict theoretically inhibition of Aflatoxin biosynthesis in fungus.

Retrieval of natural compounds

The secondary metabolites of the neem collected from various chemical structure reported research papers and the compounds were retrieved from PubChem database(https://pubchem.ncbi.nlm.nih.gov/), in this current study, we processed with 15 different chemical structures of neem tabulated in table 1.

RESULTS AND DISCUSSION

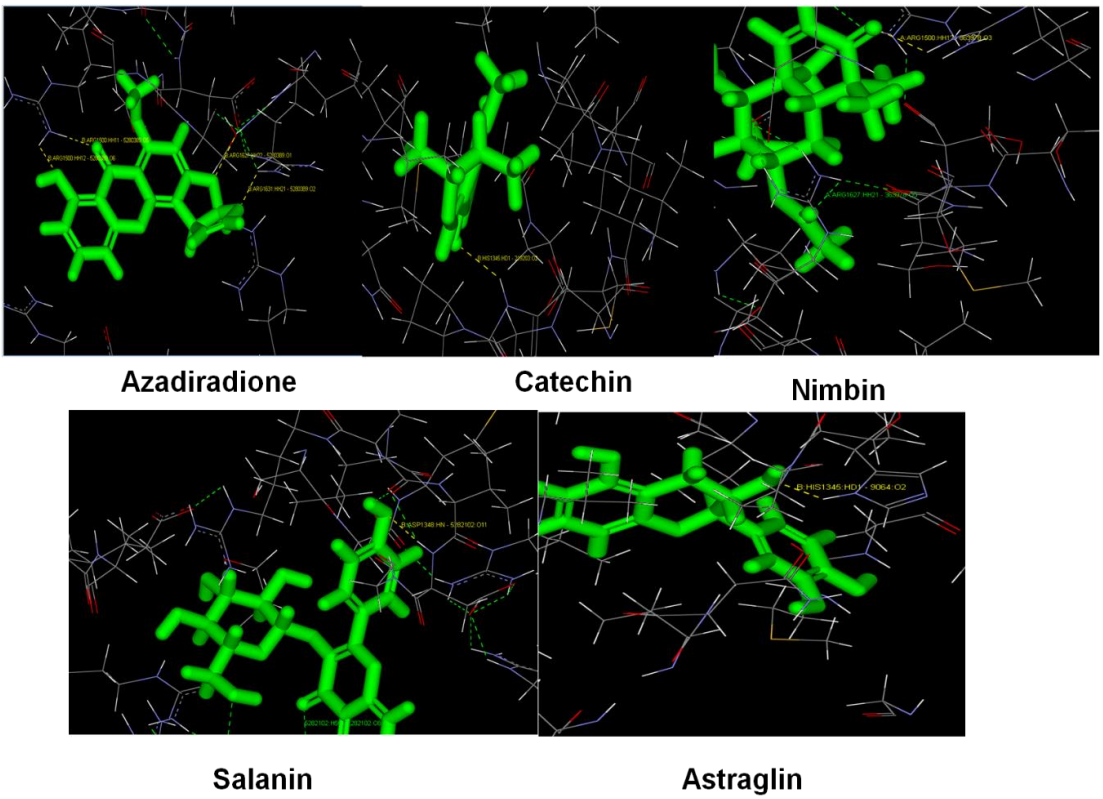

Fig 4: Interaction of enzyme active site amino acid with neem compounds

Polyketide synthase A, which initiates biosynthesis of the environmental carcinogen aflatoxin B1, is one of the multidomain iterative polyketide synthases (IPKSs), a large, poorly understood family of biosynthetic enzymes.

Table 3: Docking Results of 3HRQ with Neem Compounds

S no	Compound	Interacting amino acid residues	Distance of Hydrogen bond in Å
1	Azadiradione	B:ARG1500:HH1-Azadiradione :O5	1.199
		B:ARG1500:HH12-Azadiradione: O6	2.123
		B :ARG1631:HH21-Azadiradione :O2	1.139
		B:ARG1631:HH21-Azadiradione :O2	1.567
2	Catechin	B:HIS1345:HD1-Catechin :02	2.123
3	Nimbin	B:ARG1500:HH12: Nimbin :03	2.269
4	Salanin	B:ASP1348:HN: Salanin :01	1.872
5	Astraglin	B:HIS1345:HD1: Astraglin :02	2.391
6	Standard-Fenufluramine	B:HIS1345:HD1-Fenufluramine:F1	1.6352

Polyketides are synthesized in bacteria and fungi by a protein mega complex with multiple domains called polyketide synthase (PKS). Polyketides are synthesized in bacteria and fungi by a protein mega complex with multiple domains called polyketide synthase, it synthesizes the backbone polyketide and initiates aflatoxin biosynthesis. Hence targeting of this enzyme will reduce aflatoxin synthesis. The Polyketide synthase A with different volume of cavities based on hydrophobicity are shown in the fig 2, the variation in color from blue to brown reads the hydrophobicity of the enzyme Polyketide synthase A.

Ligand-enzyme interaction: Enzyme interaction with ligand is a crucial process in some cases the interaction may increase the process, on another case it will inhibit the process. In this current study, the secondary metabolites were acting as inhibitor to stop the biosynthesis process of aflatoxin in fungus to prevent the infection. The enzyme as different cavities, here we have chosen the largest site of volume 488.25Å and atom point count of 3906 with equal grid spacing 50 and angles 90^{o} in XYZ direction. Fig 3 and fig 4 shows the docking of ligand with active site amino acid and the following table 2 and table 3 describes about the interaction and the dock score of docked ligand with enzyme.

From the docking results it was found that the fenfluramine, has a dock score of 32.59 is attached to the protein polyketide synthase by hydrogen bonding and vanderwaals forces. The fungal protein polyketide synthase is loaded in discovery studio , the active sites of the protein polyketide synthase are found and docked with a list of name compounds. The various neem compounds such as Astragalin, Azadirachtin, Azadiradione, Azadirone, Catechin, Epicatechin,

Gallicacid, Gedunine, Kaempherol, Mahmoodin, Morgolone, Morgolonone, Nimbin, Nimbolide, Salanin.
The docking of polyketide synthase with neem compounds gives various dock scores according to their biological activity, from the list of compounds astraglin shows high biological activity against fungal protein polyketide synthase with a dock score of 42.93 interacting at residue HIS1345, the results of biological activity of neem ligands are represented .The interaction scores of this neem compounds are as follows, the dock score of polyketide synthase with azadiradione is 40.50 at residues ARG1500 and ARG1631, Cathecin with is 36.60 at residues HIS1345,Nimbin is 41.29 at residues ARG1500,Salanin is 36.71 at residues ASP 1348 and Astraglin is 42.93 at residues HIS1345.

CONCLUSION

In the current study, an attempt has been made in this review to highlight the effects of neem extracts on the malarial, bacterial and fungal toxins. Due to the production of these toxins from the microorganisms leads to affecting many food crops, humans and animals. Normally malarial, bacterial infection shows its major effect on humans and fungal infection will be more on food crops. Many synthetic drugs have worked on these, but the results of nine drugs have shown good inhibitory against fungal toxins.
The aflatoxin, which is also a mycotoxin inhibited by many artificial drugs, but due to some side effects neem compounds has been used to inhibit the production of aflatoxin in fungus. Hence, by performing molecular docking studies and results that are obtained from docking shows that the interaction with neem compounds among 15 compounds, only Astraglin shows the better interaction with the target than the standard drugs.

REFERNCES

1. Edgar J. DaSilva, EliasBaydoun, AdnanBadran. Biotechnology and the developing world.*Electronic Journal of Biotechnology* 2002;5(1).
2. Fabricant DS, Farnsworth NR.The value of plants used in traditional medicine for drug discovery. Environ. *Health Perspect* 2001; 109 (1): 69–75.
3. Dhivya S and Jaynthy C. Computational biology approach in targeting the enzyme casein II alpha subunit using triphala constituents. *International Journal of Biological & Pharmaceutical Research* 2013; 4(6):455-459.
4. El SN and Karakava S. Radical scavenging and iron chelating activities of some greens used as traditional dishes in Mediterranean diet. *Int J Food Sci Nutr* 2004; 55:67.
5. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. *J Nutr* 2003; 133(5): 1286-1290.
6. Agrawal VS and Ghosh B. Drugs plants of India (Root drugs),Kalyani Publishers, New Delhi, 1985, 1-330.
7. Fraenkel Gottfried S. The raison d'Etre of secondary plant substances. *Science* 1959; 129 (3361): 1466–1470.
8. Hartmann Thomas. From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry* 2007; 68.(22-24) :2831-2846.
9. Crozier A, Clifford MN, Ashihara H. An Overview Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet.Blackwell Publishing United States,2007,1-21.
10. Biswas, Kausik, Ishita Chattopadhyay, Ranajit K.Banerjee and Uday Bandyopadhyay. Biological activities and medicinal properties of Neem (Azadirachta indica). *Current Science* 2002;82(11): 1336-1345.
11. Yoganarasimhan SN. Medicinal Plants of India, Edn 1, Vol I, Interline Publishing Pvt Ltd, Karnataka, 1996,94-198.
12. Subapriya R and Nagini S. Medicinal properties of neem leaves: a review, Pharmacognosy Review 2004;5 (4): 408-421.
13. Bennett JW. Mycotoxins, mycotoxicoses, mycotoxicology and Mycopathologia. *Mycopathologia* 1987;100:3-5
14. Yu J, Chang PK, Bhatnagar D, Cleveland TE.Genes encoding cytochrome P450 and monooxygenase enzymes define one end of the aflatoxin pathway gene cluster in Aspergillus parasiticus. *Appl. Microbiol. Biotechnol* 2000; 53:583-590.
15. Yu JH, and Leonard TJ. Sterigmatocystin biosynthesis in Aspergillus nidulans requires a novel type I polyketide synthase. *J. Bacteriol.*1995; 177:4792-4800.
16. Yabe K, Nakamura M, Hamasaki T. Enzymatic formation of G-group aflatoxins and biosynthetic relationship between G- and B-group aflatoxins. *Appl. Environ. Microbiol* 1999; 65:3867-3872.
17. Watanabe CMH,Wilson D, Linz JE, Townsend CA.Demonstration of the catalytic roles and evidence for the physical association of type I fatty acid syntheses and a polyketide synthase in the biosynthesis of aflatoxin B1. *Chem. Biol.* 1996;3:463-469.
18. Mayorga ME. and Timberlake WE. The developmentally regulated Aspergillus nidulans wA gene encodes a polypeptide homologous to polyketide and fatty acid syntheses. *Mol. Gen. Genet*. 1992; 235:205-212
19. Feng GH.and Leonard TJ.Characterization of the polyketide synthase gene (pksL1) required for aflatoxin biosynthesis in Aspergillus parasiticus. *J. Bacteriol* 1995;177:6246-6254.