UNDERSTANDING THE MOLECULAR MECHANISM OF ACTION OF WITHANOLIDES FROM ASHWAGANDHA

SHASHANK PRAKASH KATIYAR

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

Shashank Prakash Katiyar

Department of Biochemical Engineering and Biotechnology

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This thesis is dedicated to my grandparents, who laid the foundation long back by educating my parents

The reality cannot be the Truth,

because the reality is created by perception and perception is forged

CERTIFICATE

This is to certify that the thesis entitled **'Understanding the molecular mechanism of action of withanolides from Ashwagandha'** being submitted by **Mr. Shashank Prakash Katiyar** to the Indian Institute of Technology Delhi for the award of the degree of '**Doctor of Philosophy**', is a record of the bona fide research work carried out by him, which has been prepared under my supervision in conformity with the rules and regulations of the Indian Institute of Technology Delhi. The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

Dr. D. Sundar

Associate Professor

Department of Biochemical Engineering and Biotechnology

I.I.T Delhi

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Shashank Prakash Katiyar

ABSTRACT

The plant Ashwagandha (*Withania somnifera*) is held in high repute in traditional Indian medicine system Ayurveda, largely due to the presence of withanolides such as withaferin-A, withanolide-A, withanone and many more. The withanolides are a group of naturally occurring C-28 steroidal lactones that are built on an intact or rearranged ergostane framework. Apart from withanolides, several groups of other chemical constituents such as alkaloids, flavonoids, tannin etc. have been identified, extracted, and isolated from this plant. Withanolides have diverse pharmacological properties ranging from anti-tumor, anti-inflammatory, anti-stress, anti-oxidant, immuno-modulatory, hemopoetic, cardio-protective and neuro-protective in nature. Because of these properties, Ashwagandha has been compared to ginseng and thus is also referred to as Indian ginseng.

Recently, withanolides have gained importance for testing on cancer cells and they are found to affect the functional activity of cancer-related proteins. Not only the potential of withanolides against cancer cells makes these natural compounds a candidate of interest, but also the recent reports of their selectivity against cancer cells, demands an urgent need to explore the potential of withanolides and the basis of such selectivity. Though the therapeutic effects of these withanolides are well documented, the detailed specific action-mechanisms on the respective disease-associated targets are still largely unknown. The focus of the research in this thesis was to elucidate the mode of action of withanolides on various crucial therapeutic targets using computational techniques. The mechanism of action of withanolides has been investigated using computer simulations and attempts were made to conduct experimental validations as well. The modes of selectivity towards cancer cells by withanolides at the molecular level has also been studied in detail. The permeable nature and metabolic fate of the withanolides to understand their drug-like potentials has also been explored. In the process of understanding the mechanism of withanolides, molecular mechanisms of proteins and their interactions with other natural molecules have also been documented.

Overall, the research focus of this PhD project was directed towards broadening our understanding of the action mechanism of withanolides by exploring their interactions with biological targets and validating their effect on different pathways.

सारांश

अश्वगंधा के पौधे का पारंपरिक भारतीय चिकित्सा प्रणाली में मुख्यतः विथानोलाइड, जैसे की विथफेरिन-ए, विथानोलाइड-ए, विथानोन, एवं अन्य कई विथानोलाइड की वजह से उच्च स्थान है। विथानोलाइड प्राकृतिक रूप से पाये जाने वाले २८-कार्बन से बने स्टेरॉइडल लैक्टोन हैं, जोकि स्थिर तौर पर बरक़रार रहने वाले या पुनर्व्यवस्थित होने वाले एर्गोसटोन की रूपरेखा पर बने होते हैं। इस पौधे में विथानोलाइड के अलावा भी कई अन्य रासायनिक घटक जैसे कि अल्कलॉइड, फ्लैवोनॉइड, टैनिन पहिचाने एवं अलग किये गए हैं। इस पौधे में विथानोलाइड के अलावा भी कई अन्य रासायनिक घटक जैसे कि अल्कलॉइड, फ्लैवोनॉइड, टैनिन पहिचाने एवं अलग किये गए हैं। विथानोलाइड में एंटी-ट्यूमर से लेकर एंटी-स्ट्रेस, एंटी-ऑक्सीडेंट, इम्मुनोमोड्यूलेटरी, हेमोपोइटिक, कार्डियो-प्रोटेक्टिव, एवं न्यूरो-प्रोटेक्टिव तक भांति प्रकार की औषधीय गुण पाये जाते हैं। इसी प्रकार के गुणों की वजह से, अश्वगंधा की तुलना जिनसेंग से की जाती है और इसी कारणवस यह भारतीय जिनसेंग कहलाता है।

हाल ही में, विथानोलाइड की महत्वपूर्णता को कैंसर कोशिकाओं पर परिक्षण की वजह से बढ़ावा मिला है और वे कैंसर से जुड़े हुए प्रोटीनों की कार्यात्मक गतिविधि को प्रभावित करते हुए पाए गए हैं। सिर्फ कैंसर कोशिकाओं के विरुद्ध विथानोलाइडों की क्षमता ही इन प्राकर्तिक यौगिको को रिचिपूर्ण नहीं बनाता,बल्कि हालही में इनकी चयनकता के बारे में आयी सूचनाएं इनकी अन्य क्षमताओ की खोज की एवं इस प्रकार के चयनिक क्षमताओ के आधार की खोज की तात्कालिक मांग करती हैं । यद्यपि, इन विथानोलाइडों के उपचारात्क प्रभाव अच्छी तरह से प्रलेखनित हैं, इनके विस्तारपूर्ण रोग सम्बंधित विशिष्ट क्रिया-तंत्र का वर्णन अभी भी अज्ञात है। इस शोध का केन्द्रीकरण, अभिकलनात्मक पद्धितियों के द्वारा विथानोलाइडों की विभिन्न महत्वपूर्ण चिकित्सीय लक्छओं की क्रियाओं के माध्यम की विस्तृत व्याख्या करना था। विथानोलाइडों की क्रिया पद्धितियाँ अभिकलनात्मक अनुकरणों के द्वारा निरूपित की गई हैं एवं प्रयोगों के द्वारा पुष्टिकरण करने का प्रयास भी किया गया है। पुष्टिकरण करने का प्रयास भी किया गया है। विथानोलाइड द्वारा कैंसर कोशिकाओं के प्रति चयनकता भी विस्तार से निरूपित की गई है। विथानोलाइड की पारगम्य प्रकृति एवं मेटाबोलिक भाग्य को भी इनके औषधी बनाने की संभावना को समझने हेतु खोजा गया है। विथानोलाइड की यांत्रिक क्रिया को समझने की प्रक्रिया मे, कई प्रोटीनों की यांत्रिक क्रिया एवं उनके अन्य प्राकृतिक घटकों के साथ पारस्परिक संबंधों को भी निरूपित किया गया है।

सम्पूर्णतः, इस शोध पत्र का केंद्र, विथानोलाइडों के साथ जैविकीय लक्छ्यों के संबंधों की जानकारी से एवं उनके प्रभावो का कई पाथवे पर अध्ययन के द्वारा विथानोलाइडों के क्रिया तंत्र के बारे में अपनी समझ को बढ़ने की दिशा में था।

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LIST OF ABBREVIATIONS AND ACRONYMS

AIST	The National Institute of Advanced Industrial Science and Technology
ALT	Alternate mechanism of lengthening of Telomeres
APL	Area per lipid
COM	Center of mass
DAILAB	DBT-AIST International Laboratory for Advanced Biomedicine
DBD	DNA binding domain
hnRNP-K	Heterogeneous nuclear riboprotein-K
HSP90	Heat-shock protein 90
L PKC	Leishmanial protein kinase C
MD	Molecular dynamics
MRN	MRE11-RAD50-NBS1
$p53^{143A}$	V143A p53 mutant
$p53^{220C}$	Y220C p53 mutant with an inhibitor
$p53^{249S}$	R249S p53 mutant
$p53^{273C}$	R273C p53 mutant
$p53^{273H}$	R273H p53 mutant
$p53^{w}$	Wild type p53
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
ROG	Radius of gyration
SASA	Solvent accessible surface area
SMD	Steered molecular dynamics
SP	Standard precision
TA	Tubocapsanolide-A
TEP	Telomerase plus
$^{u}p53^{220C}$	Unbound Y220C mutant
Wi-A	Withaferin-A
WN	Withanone
XP	Extra precision