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Short Communication

Metabolomic insights into plant-derived cyclolinopeptides as natural therapeutics for management of paroxysmal hypertension: an *in-silico* approach

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ABSTRACT

Paroxysmal hypertension is a type of response exerted by the body towards external stimulus like physical, mental, or emotional stress which leads to chemical changes in the body that accelerates the physiological parameters like stress hyper-glycemia, obesity, stress cardiomyopathy, hemorrhagic stroke etc. Due to ill effects of chemical drugs administered by the clinicians, there is need of natural anti-depressant products which may serve as an alternative medicine. Compounds like cyclolinopeptides, cyclosporin A, and curcacyclines have exhibited potential immunosuppressive activity. With objective of drug discovery, this study investigates the role of sub-types of cyclolinopeptides as potential drugs against paroxysmal hypertension and predict their mode of action in biochemical pathways and their role in mitigation of stress induced hyper response.

Keywords: Cyclolinopeptides, Metabolic pathway, Molecular docking, Na+/K+ pumps, Paroxysmal hypertension

INTRODUCTION

Stress is defined as a type of natural and evolutionary *"fight or flight"* response comprising of physical, mental, and emotional reactions to an external stimulus.[1-3] Such studies provide strong insight of correlation between paroxysmal hypertension and stress leading to other diseases like hypo-immune dysfunction, stress hyper-glycemia, obesity, stress cardiomyopathy hemorrhagic stroke and having greater vulnerability towards stress-induced asthma (SIA), atopy etc.[4] Among all, suboptimal blood pressure control has been identified as the third ranked factor for disability-adjusted life years. Proteins highly expressed during stress include-the mineralocorticoid receptor, insulin receptor, glycogen synthase kinase3 receptor and aldose reductase receptor are intrinsically correlated with blood pressure regulation, glucose regulation and many more functions.[3-7] Other than this protein mineralocorticoid receptor play essential role in electrolyte and fluid homeostasis whereas insulin receptor plays key role in glucose homeostasis that under clinical conditions may result in stress related diseases like hyperglycemia and hyperglycemia, respectively.[4,5,8] Medicines administered by physicians relieve stress by inducing sleep, but they are not intended for long-term use because they have a number of detrimental side effects such as burning sensations, dizziness, etc. These medicines either hinder the stress hormone receptors or they directly regulate the hormones. While other body responses like increasing blood pressure and blood glucose are not regulated by such medicines. These

proteins play vital roles in maintaining balance in fluid homeostasis and glucose homeostasis that under clinical conditions may result in stress related diseases like hyperglycemia and paroxysmal hypertension, respectively.[8-10]As a result, there is need to generate a library of phytocompounds that can enable regulation and deregulation of proteins responsible for management of paroxysmal hypertension as well as studies predicting their mode of action. This study will be focused on in-silico identification of potential drugs validated by pathway prediction to suggest phytocompounds as alternate medication for the treatment of paroxysmal hypertension that could be used as drugs after in-vivo studies and clinical trials and later can be produced by the pharmaceutical industry.[11-34]

As "superfood," flaxseeds are widely regarded as a safe (GRAS) source of nutrients such vitamins, minerals, proteins, peptides, lipids, omega-3 and omega-6 polyunsaturated fatty acids, carbs, lignans, and dietary fibre. Cyclolinopeptides (CL), are a group of naturally occurring hydrophobic cyclic peptides found in flaxseed (*Linumusitatissimum* L).[14] In folk medicine, flaxseed has been used to reduce the pain caused by traumatic injuries and arthritis or skin inflammations. Studies on cyclolinopeptides have confirmed its natural therapeutic qualities as antioxidant, anti-inflammatory, antihypertensive, immune suppression/enhancement, glucose absorption control and immunosuppressive activity.[13] This work aims to validate the role of cyclolinopeptide as natural inhibitors for regulation of paroxysmal hypertension and other related diseases in support of theory of cyclolinopeptides as stress regulators for balancing out the normal biological conditions. This study was based on two objectives (a) Identification of novel drug target for Paroxysmal Hypertension. (b) Prediction of molecular pathway revealing role of cyclolinopeptides in management of paroxysmal hypertension via selected drug target. AutoDock 4.2.0 tool was employed for investigation of detailed intermolecular interactions of ligands with the biomolecular targets, whereas prediction of molecular pathway was performed via KEGG database.[14,15]

METHODS

Study design and period

This *in-silico* study was conducted from January 2022 to June 2022. The aim of the study was to explore the metabolomic insights into plant-derived cyclodipeptides as potential natural therapeutics for the management of paroxysmal hypertension.

Molecular docking

The crystal structure of 13 diverse human proteins determined using X-ray diffraction method are given in Table 1, these proteins were retrieved from protein data bank in pdf format. The preparation of protein molecules was performed by BIOVIA Discovery Studio Visualizer application.[9] cyclolinopeptides (CLA-CLI) reported to have immunosuppressive activity in various literature were retrieved using the KnapSack3D database in mol file format. The saved ligand compound structures were optimized and converted to PDB format in Open Babel software. The molecular docking was carried out using Lamarckian Genetic Algorithm in AutoDock 4.2.0.

Table 1: List of selected proteins from PDB involved in stress induced paroxysmal hypertension and cyclolinopeptides with immunosuppressive activities.

S. No.	Types of proteins			
A	**Protein receptor**	**PDB Id**	**Resolution**	**Ref.**
1.	GLP-1 receptor	3C59	2.3 Å	18
2.	AMPK (AMP-activated protein kinase receptor)	4CFH	3.24 Å	19
3.	GSK3 (Glycogen synthase kinase 3)	1H8F	2.80 Å	20
4.	PPAR (Peroxisome proliferator activated receptor gamma)	2ZK0	2.36 Å	21
5.	FFAR1 (Free fatty acid receptor)	5TZR	2.20 Å	22
6.	HPA (Human pancreatic alpha-amylase)	1HNY	1.80 Å	23
7.	Aldose reductase	3S3G	1.80 Å	24
8.	Human pancreatic phospholipase A2	3ELO	1.55 Å	25
9.	Mineralocorticoid receptor	4TNT	2.39 Å	21
10.	GLUT-4 (Glucose transporter 4 receptor)	3PCU	2 Å	26
11.	HNF-1α (Hepatocyte nuclear Factor 1 alpha)	3CBB	2 Å	27
12.	IA-2 (Insulinoma-associated protein 2 receptor)	2QT7	1.30 Å	28

Continued.

S. No.	Types of proteins			
13.	Insulin receptor	1IR3	1.90 Å	10
B	**Cyclolinopeptides type**	**Molecular formula**	**Molecular weight (Da)**	**Ref.**
1.	CLA	$C_{57}H_{85}N_9O_9$	1040Da	29
2.	CLB	$C_{56}H_{89}N_9O_9S$	1058Da	30
3.	CLC	$C_{56}H_{83}N_9O_{10}S$	1074Da	31
4.	CLD	$C_{57}H_{77}N_9O_9S$	1064Da	27
5.	CLE	$C_{51}H_{77}N_8O_9S$	977Da	31
6.	CLF	$C_{55}H_{73}N_9O_{10}S_2$	1084Da	32
7.	CLG	$C_{56}H_{75}N_9O_{10}S_2$	1098Da	32
8.	CLH	$C_{56}H_{75}N_9O_9S_2$	1082Da	15
9.	CLI	$C_{55}H_{73}N_9O_9S_2$	1068Da	15

Pathway prediction

To investigate the molecular pathway of mineralocorticoid receptor mediated stress response, cascades involving mineralocorticoid receptor expression was identified by KEGG database and its role in management of paroxysmal hypertension was predicted using the same (https://www.genome.jp/kegg/). Pathway identification parameters for query search were, "molecule: mineralocorticoid receptors and origin: homo sapiens" and pathway involving 'mineralocorticoid receptor' was selected.

RESULTS

Molecular docking

As a result of ADT calculations, different conformations of the protein-ligand complex were obtained. Each structure has been scored and ranked in Table 2, based on the calculated Gibbs free energy generated during interaction.[30] Docking is used to anticipate the binding mechanism and affinity of new binders and to optimize current medicines. Moreover, the speed of these techniques enables virtual screening of ligand libraries including tens of thousands of different molecules.[33]

From the docking analysis between cyclodipeptides as ligands and stress responsive proteins, Mineralocorticoid receptor (4 TNT) showed best binding affinity to the ligand CLE-4 TNT (ΔG=-3.31 kcal/mol) and lowest binding energy was shown by CLG-3ELO (ΔG=2.31 kcal/mol) respectively.

Docking poses of complexes possessing highest and lowest binding energy are given in Figure 1a and Figure 1b.

Table 2: Binding energy of docked ligand cyclolinopeptides with different target proteins.

S. no.	Receptor	Ligand	Amino acid residue	Binding energy (Kcal/mol)	H bond length
1.	IH8F	CLI	TYR146	-0.91	2.222
2.	1HNY	CLC	THR163	-1.12	2.135
3.	1IR3	CLD	ARG1155	1.47	2.261
4.	2QT7	CLC	GLN103	-2.86	2.096
5.	2ZK0	CLC	LYS358	-1.18	2.199
6.	3C59	CLA	SER32	-2.01	2.152
7.	3CBB	CLC	GLN122	-1.33	1.928
8.	3ELO	CLG	ASP6	2.31	2.041
9.	3PCU	CLA	THR278	-1.12	2.149
10.	3S3G	CLI	ARG268	-0.66	2.232
11.	4CFH	CLE	LYS60, ARG63, ARG63	-2.37	1.921, 1.874, 2.063
12.	4TNT	CLE	LYS653, TYR637	-3.31	2.055, 1.817
13.	5TZR	CLG	ARG1080	1.46	2.019

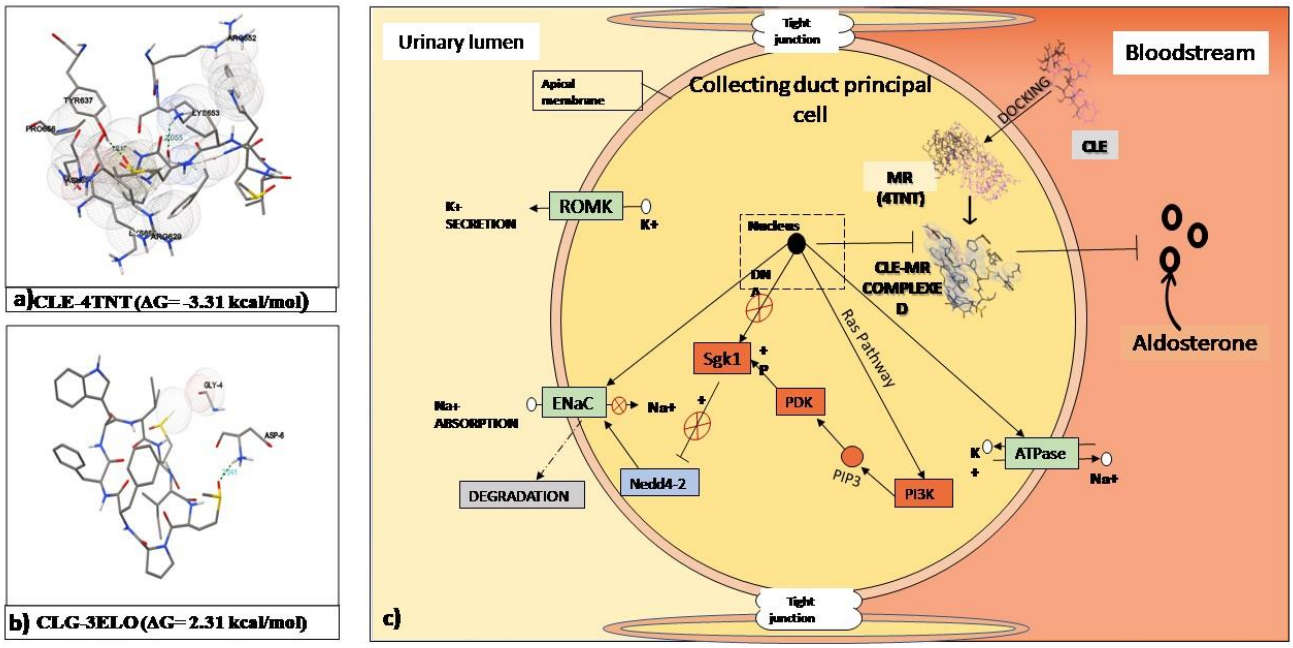

Figure 1: This diagram shows the docking pose of cyclolinopeptide giving best and least binding energy with stress responsive protein. by Mineralocorticoid receptor in paroxysmal hypertension.

a) Mineralocorticoid receptor (PDB Id- 4TNT) with CLE (ΔG= -3.31 kcal/mol). b) Human pancreatic prophospholipase A2 (PDB Id- 3ELO) with CLG (ΔG= 2.31 kcal/mol). c). This diagram depicts the molecular pathway involved in stress related response mediated.

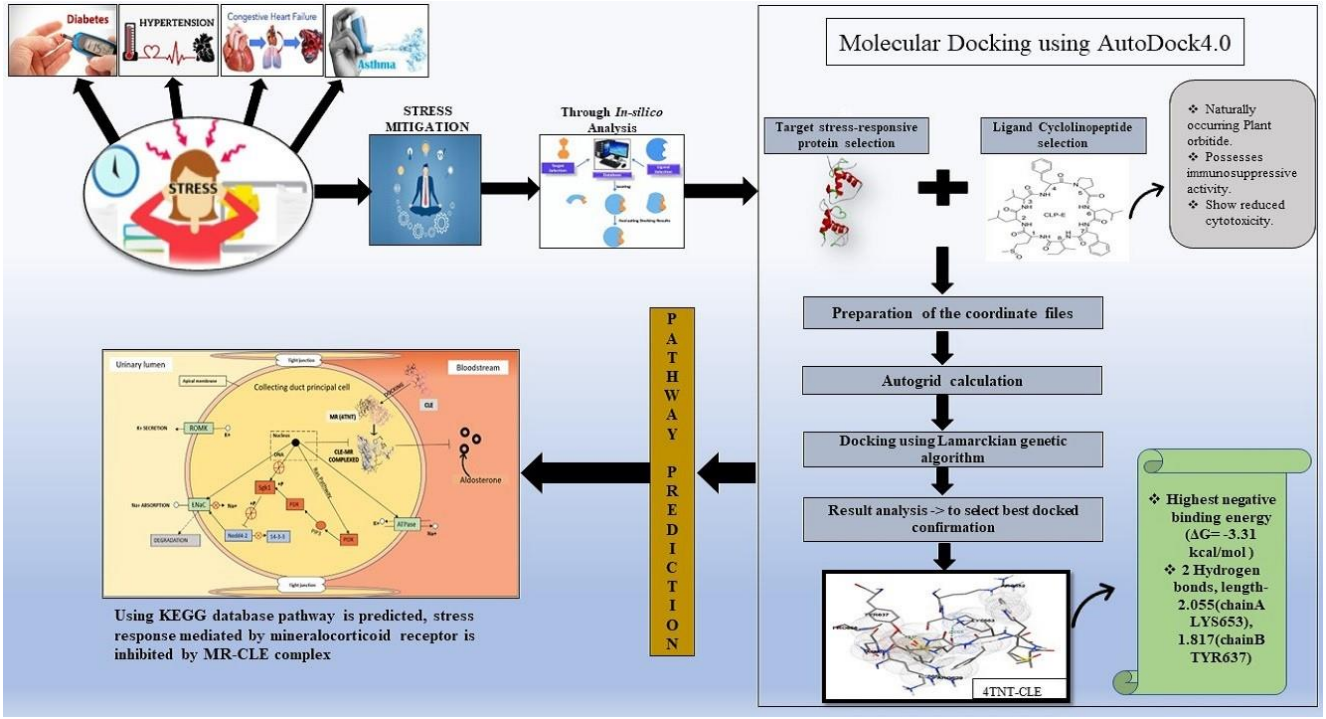

Figure 2: Graphical abstract.

Limitations

This study has certain limitations that should be acknowledged. First, being an in-silico study, the findings are based on computational models and simulations, which may not fully capture the complexity of biological systems in vivo. Second, the lack of experimental validation in biological systems means that the therapeutic potential of cyclolinopeptides observed here needs to be confirmed through in vitro and in vivo studies. Finally, the scope of metabolomic data available for analysis was limited, which may have restricted the

comprehensiveness of the metabolic pathways and interactions identified. Despite these limitations, the study provides valuable insights and a foundation for future research into plant-derived cyclolinopeptides as natural therapeutics.

CONCLUSION

The *in-silico* study validates the potential and mechanisms for modulating protein expression associated with stress-induced diseases like paroxysmal hypertension. Utilizing Autodock 4.2.0 for molecular docking, we found that cyclolinopeptide E (CLE) exhibits the highest negative binding energy with the mineralocorticoid receptor, a crucial stress-responsive hormone receptor. Our findings suggest that CLE effectively mitigates stress responses by preventing excessive Na+ ion reabsorption, which is mediated through the deactivation of the mineralocorticoid receptor. This interaction disrupts aldosterone's ability to activate Sgk-1, leading to the proteasomal degradation of ENaC channels and ultimately reducing hypertension. Additionally, the study highlights the interplay between the mineralocorticoid receptor, Na+/K+ pumps, and insulin absorption mechanisms, elucidating the link between stress, hypertension, and hyperglycemia. The research supports the utility of cyclolinopeptides as natural antihypertensive agents with minimal side effects, unlike conventional drugs. This research not only fills critical gaps in understanding the molecular mechanisms of stress-induced hypertension but also sets a foundation for future in-vivo and in-vitro validations to determine optimal dosages for effective treatment.

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All authors contributed to the study conception and design. Ruchi Khare did conceptualization, drafted the definition of intellectual content and performed review writing of the manuscript. Material preparation, data collection, extensive data curation and review writing were performed by Ms. Akanksha Mishra, Dr. Rahul Shrivastava made critical revisions, analyzed manuscript, and supervised throughout the process. All authors read and approved the final manuscript. We acknowledge Maulana Azad National Institute of Technology for providing infrastructure required to conduct the study.

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