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N Roy
Distributed Information Centre (DIC), Kerala Agricultural University (KAU), Thrissur, Kerala, India

PA Nazeem
Distributed Information Centre (DIC), Kerala Agricultural University (KAU), Thrissur, Kerala, India

PS Abida
Centre for Plant Biotechnology and Molecular Biology (CPBMB), KAU, Thrissur, Kerala, India

Correspondence
N Roy
Distributed Information Centre (DIC), Kerala Agricultural University (KAU), Thrissur, Kerala, India

Comparative docking studies to prove the accuracy of computational tools for recognizing the inhibitory action of garlic (*Allium sativum* L.) on diabetes

N Roy, PA Nazeem and PS Abida

Abstract
Diabetes mellitus (DM) is a major metabolic disease occurring worldwide both in developed and developing countries. It is symptomized with hyperglycemia and insufficiency in insulin secretion and action. There are several therapeutic targets present in our body, the overexpression or dysregulation of which provokes inception of DM. Garlic (*Allium sativum* L.) is one of the most widely used spice crop as a popular remedy for various ailments and disorders due to the presence of bioactive organosulfur compounds (OSCs). The objective of the present study was to check the accuracy of *in silico* tools in re-establishing the inhibitory activity of most common garlic OSCs on those targets through molecular docking (MD) studies and also evaluate drug likeliness of the best OSCs through pharmacokinetic analysis. These analyses were conducted using Discovery Studio (DS) software version 4.0. MD studies revealed four OSCs from garlic which inhibit the druggable targets thus highlighting their action over health.

Keywords: Diabetes, therapeutic targets, garlic, organosulfur compounds, *in silico*, molecular docking, Discovery Studio

Introduction
Delivery of glucose to the cells in the body is ensured by the contrary action of two hormones insulin and glucagon. Duty of these hormones is assisted by enzymes such as Aldose Reductase (AR), Dipeptidyl Peptidase-4 (DPP4) and Glycogen Synthase Kinase 3 (GSK3) and maintains a complex signalling system. There may emerge some abnormality or impairment in the regulation of these enzymes, which results into serious complications relating to DM [1, 2, 3]. According to International Diabetes Federation (IDF), there are currently 415 million people diagnosed with diabetes and the total is expected to rise to 640 million by 2040 [4]. Similarly, our country India as predicted by IDF will have 100 million people with diabetes by the same time [5]. Many synthetic medications are available in market against DM, but prolonged use of them can cause serious health side effects [4]. Plant compounds are always been in limelight as they have reduced toxicity and side effects compared to synthetic compounds. Garlic contains many OSCs which warrants novel research to exploit their potential. Studies related to diabetes, where garlic and garlic extracts showed effectiveness in reducing insulin resistance was already reviewed [6, 7]. *In silico* MD tool from DS 4.0 software was used in the present study to know the binding affinity of the garlic OSCs with the DM target proteins, thus re-establishing the action of garlic over diabetes [2, 8].

Materials and methods
Retrieval of target proteins and active site (AS) selection
The 3D crystallographic structures of successful targets for DM such as AR (PDB ID: 1IEI complexed with zenarestat), DPP4 (3W2T with vildagliptin), and GSK3 (1Q41 with Indirubin-3'-monoxime) were retrieved from Protein Data Bank (PDB) with a resolution ranging from 2.1 to 2.5 Å [8]. These protein structures were pre-processed for making them ready for docking following all the steps as prescribed by Glaab, 2016 and finally made into a stable conformation after energy minimization by CHARMM22 (Chemistry at Harvard Macromolecular Mechanics) force field [8]. The co-crystallized ligands were removed from the protein structures [1].

ASs were identified using poseview software molecular interactions of protein target crystal structures and co-crystallized ligands displayed in PDB [9]. A grid box was prepared to hold the critical amino acids in the binding site (BS), and make the docking of the molecules to take place within the BS.

Retrieval, pre-processing, and filtering of OSCs

3D structures of the most common OSCs [Allicin 65036, Alliin/S-allyl cysteine sulfoxide (SACS) 87310, Allyl mercaptan 13367, Methyl propyl disulphide 16592, S-allyl-L-cysteine (SAC) 98280, S-allyl-mercapto cysteine (SAMC) 9794159, Diallyl di- sulphide 16590, Dimethyl disulphide 16590, Dimethyl difuran 236769, Allyl methyl disulphide 62434, Allyl propyl disulphide 16591, L-γ-glutamyl-S-allyl-L-cysteine (γGSAC) 91820320, Thiacremonone 539170, 2-vinyl-4H-1,3-dithiin 133337 and E-ajoene 5386591] were retrieved from PubChem database [8]. These ligand structures were pre-processed by removing duplicates, listing of tautomers/isomers, abolish intra-magnetism, adding hydrogen bonds (HB) and energy minimized by CHARMM22 force field. Ligands were also prefiltered by Lipinski's Rule of five and Vebers' protocol (Ro5 & VP) for evaluation of their drug likeliness [2, 8].

Molecular docking, binding energy calculation and pharmacokinetic evaluation

Structure based MD was performed between DM target proteins and the OSCs by 'CDOCKER' protocol of DS 4.0 to know the binding affinity [10]. The docking procedure was repeated thrice to reconfirm the result. A total of 10 conformations were allowed to be obtained. The scoring function was analysed by HB formation among the molecules using Binding Energy (BE) calculation. The conformation with lowest BE is considered as the best interaction [2]. Next the interacting ligands were subjected to ADMET (absorption, distribution, metabolism, excretion and toxicity) parameters inspection to evaluate whether these have the potential to be candidate drugs [2, 8].

Comparative studies

Interaction efficiency of the OSCs with the DM targets was compared by allowing marketed drugs to interact with the same DM targets. Three separate commercial drugs were selected as true inhibitors of the target proteins, identified from Drugbank [11]. The drugs selected were tolrestat for AR, linagliptin for DPP4 and lithium carbonate for GSK3.

Results and discussion

To make proper inhibition by ligands, selection of perfect AS is necessary as proteins contain multiple ASs [2, 8]. Likewise, no. of AS contained in each protein structure: AR=2, DPP4=17 and GSK3=2). But from all of the structures AS no. 1 (AC1) was chosen as the BS because these BSs were the sites where the co-crystallized ligands deposited in PDB were bound [1, 10]. Prefiltering of 15 ligands by Ro5 & VP on parameters with cut-off values like molecular weight (MW, ≤500 daltons), logP value (≤5), no. of hydrogen bond donors (HBD, ≤5), hydrogen bond acceptors (HBA, ≤10), sum of HBD and HBA ≤12, no. of rotatable bonds (≤10), polar surface area (PSA, ≤140 $Å^2$) revealed that all of the OSCs possess general drug like properties [2]. But only 4 OSCs (SACS, SAC, SAMC and γGSAC) showed interaction with the DM targets at the preferred BS (Table 1). The HBs formed between the targets and compounds were all of length <2.4 Å which indicated very good interaction [9, 10]. The lowest BE was shown by γGSAC when docked with DPP4 in 3rd run. DPP4 was the most successful target which had interaction with all the 4 docked compounds. The unprepared 3D structure of DPP4, its energy minimized stable (prepared) structure along with its AS containing all the amino acid residues and the docked complex of SAC with DPP4 is shown in Figure 1.

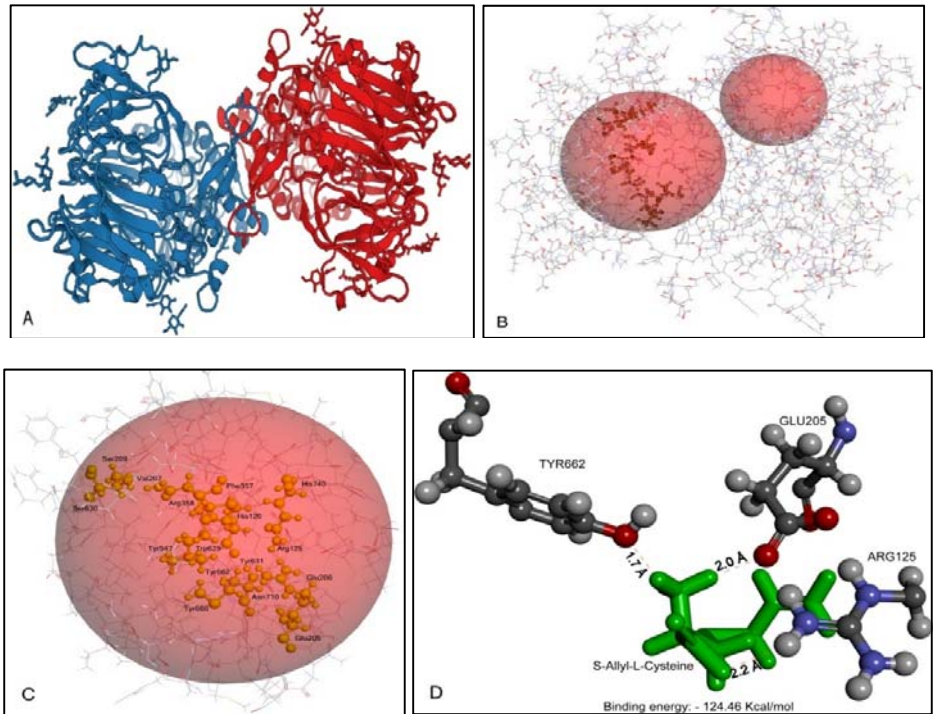

Fig 1: (A) Unprepared 3D structure of DPP4 (3W2T) from PDB, (B) Selection of AS (big sphere), (C) Amino acid residues in the AS, (D) Docked complex of SAC with DPP4.

Comparative studies with commercial drugs revealed that though they were able to bind with their respective targets at the preferred BSs, they displayed a low BE score than the OSCs and less no. of HBs were formed than that formed by the OSCs. Also the length of HBs formed by the commercial drugs with the targets exceeded the general distance of 2.4 Å which indicated weak interaction (Table 1).

Table 1: Dock scores of binding garlic OSCs and commercial drugs with the DM target proteins

Type of compound	Target Proteins	BE (Kcal/mol) 1st run	BE (Kcal/mol) 2nd run	BE (Kcal/mol) 3rd run	Interacting residues	No. of HBs	HB length (Å)
Garlic OSCs							
γGSAC	DPP4	-290.54	-288.77	-293.93	GLU206 (2), GLU205, ARG125 (2), TYR547, HIS740	7	2.4, 2.0, 1.7, 1.9,1.6, 2.2, 2.3
SACS	AR	-226.66	-229.65	-227.71	LYS21, SER210 (2), SER214 (2)	5	2.4, 2.2, 1.9, 1.7, 1.5
	DPP4	-154.09	-150.74	-155.49	TYR662, GLU205, GLU206 (2), ASN710	5	1.9, 2.0, 2.0, 2.3, 1.7
	GSK3	-111.6	-113.86	115.92	TYR134, VAL135, PRO136	3	1.2, 1.4 1.9
SAC	AR	-79.82	-82.57	-76.86	SER210, SER214	2	2.2, 1.8
	DPP4	-124.46	-120.99	-122.33	TYR662, GLU205, ARG125	3	1.7, 2.0, 2.2
	GSK3	-85.05	-82.31	-87.43	VAL135, TYR134, PRO136	3	2.3, 1.6, 2.4
SAMC	DPP4	-126.87	-123.59	-127.64	TYR547, ASN710 (2), TYR662, SER630	5	1.5, 1.9, 2.0, 1.7, 1.8
	GSK3	-145.6	-143.87	-140.88	TYR134 (2)	2	2.0, 2.3
Commercial drugs							
Tolrestat	AR	-60.35	-61.44	-58.67	LYS262	1	2.6
Linagliptin	DPP4	-88.84	-85.83	-90.53	TYR662	1	2.8
Lithium carbonate	GSK3	-59.31	-57.32	-62.26	TYR134	1	2.5

When ADMET properties (Table 2) of the 4 OSCs were checked, it was revealed that 3 OSCs γGSAC, SACS and SAC showed a little more solubility in water because of the lower logP value and low MW depicting low lipophilicity. Higher lipophilicity increases the chance of active efflux process thus reducing bioavailability of a compound [1, 2]. So, all these 3 OSCs will have high bioavailability inspite of more solubility in water. For the same 3 OSCs, the intestinal absorption was calculated as poor, but these OSCs were simultaneously found having PSA score less than 120 $Å^2$ (except γGSAC) which indicated that they generally have favourable intestinal absorption. γGSAC while interacting with DPP4 made an abnormal increase in number of HBs which resulted in considerably lowered absorption [8]. Other than these parameters, all the 4 OSCs were observed to have no hepato-toxicity and are non-inhibitors of CYP450 enzyme i.e. there is no chance of unwanted drug-drug interactions. The plasma protein binding (PPP) parameter predicted that all the OSCs have ability to get unbound from plasma proteins to perform pharmacological activity when needed. The blood brain barrier (BBB) level of all the OSCs was calculated as low and hence, there is less chance of side effects for the central nervous system [9, 10].

Table 2: Pharmacokinetic Assessment of garlic OSCs

Sl. No.	Ligand	Pubchem ID	MW (≤500 daltons)	Solubility level (2-4)	Absorption level (0-1)	Hepatotoxic Prediction (False-non toxic)	BBB level (2-4)	CYP2D6 Prediction (False-non inhibitor)	PPB Prediction (False-poorly bound)	logP (≤5)	PSA (≤140 $Å^2$)
1.	γGSAC	91820320	290.33	5	3	False	4	False	False	-4.51	162.3
2.	SACS	87310	177.22	5	3	False	4	False	False	-3.39	104.05
3.	SAC	98280	161.22	5	3	False	4	False	False	-2.28	93.07
4.	SAMC	9794159	193.28	4	0	False	3	False	False	-0.48	120.97

Conclusion

The present study focused on the validation of computational studies for re-establishing the effect of garlic on diabetes and hence it was proved. MD studies thus clarified the appropriate binding mode of OSCs with the druggable targets. Pharmacokinetic assessment of the OSCs revealed no violation of Ro5 & VP i.e. they are possessing drug like properties. The OSCs also have no toxicity, no drug-drug interaction problem and low BBB penetration, indicating their benefits for human use.

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