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Original article

In silico investigation on alkaloids of *Rauwolfia serpentina* as potential inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase

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ABSTRACT

Present work aimed to investigate the *in silico* activity of the alkaloids of roots of *Rauwolfia serpentina* as inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR). For this purpose, the three-dimensional (3D) structure of the protein HMGCR (PDB ID: 1HW9) was downloaded from Protein Data Bank (PDB) database, as a target enzyme. The structures of twelve alkaloids from the roots of *R. serpentina* were selected as ligands and docked with the selected HMGCR enzyme using Molegro Virtual Docker (MVD) software. The software 'MVD' computes the binding (atom) energies of selected protein (enzyme) and each ligand at minimum energetic conformation state by using the PLP (Piecewise Linear Potential) scoring mechanism. Docking results of twelve tested alkaloids showed that five alkaloids including compound 1 (ajmalicine), 2 (reserpine), 3 (indobinine), 4 (yohimbine), and 5 (indobine) have displayed the highest MolDock scores and best fit within the prominent active site residues (positioned between 684 and 692 of *cis-loop*) of HMGCR. According to the lowest MolDock energies obtained through non-covalent interactions of alkaloids with HMGCR, these are characterized to be the potential inhibitors of HMGCR. Therefore, the alkaloids from *R. serpentina* can effectively suppress the cholesterol biosynthesis pathway through inhibition of HMGCR and can serve as potential lead compounds for the development of new drugs for the treatment of hyperlipidaemia.

1. Introduction

High risk of cardiovascular disease (CVD), stroke as well as peripheral vascular disease are mainly linked with hypercholesterolemia, which has also been associated with high blood pressure and diabetes (Gholap et al., 2011; Balakumar et al., 2016; Lonardo et al., 2018). The gradual accumulation of steroid plaque on the walls of coronary arteries (atherosclerosis) due to the elevated blood cholesterol level actually induced danger for heart tissues (Hajar, 2017) by narrowing and hardening of surrounding of arteries which ultimately reduces the blood flow towards heart, resulting in angina (chest pain) or myocardial infarction (Joshi et al., 2015). The biochemical basis of hypercholesterolemia is absolutely or relatively due to the enhanced cholesterol biosynthesis which mainly depends on highly-expressed activity of 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCR) (Istvan and Deisenhofer, 2001; Cerqueira et al., 2016). The reductase controls the status of mevalonate and other isoprenoids precursor(s) thereby regulating the yield of cholesterol in the body (Cerqueira et al., 2016). This enzyme is inhibited/suppressed either by high amount of cholesterol through feedback mechanism, or its dephosphorylation induced by hormones (glucagon and glucocorticoids) or degradation of LDL-c (low density lipoprotein-cholesterol) via up-regulating its receptors in the body (Nes, 2011; Cerqueira et al., 2016). In this regard, competitive inhibitors of this reductase like commercially available cholesterol-decreasing group of medicines "Statins" prompt the manifestation of LDL-c receptors in hepatic tissues, thereby increasing the plasma LDL-c catabolism, lowering the circulating cholesterol concentration and reducing the risk and consequences of hypercholesterolemia (Istvan et al., 2000; Cerqueira et al., 2016). Therefore, this enzyme is considered as the prominent biochemical target of medicinally important inhibitors to reduce the chances of cardiac problems (Koskinas et al., 2018).

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Proteins, the functional blocks of life, are assembled as repetitive chains of amino acids, which then folded into distinctive 3D shapes (DuBay et al., 2015). Protein sequences are significant for bioinformatics based studies because they allow to study the naturally occurring well-arranged amino acids composition of a protein to study proteins as the key resource of information (Diniz and Canduri, 2017; Baxevanis et al., 2020). Bonding pattern within protein molecules is the key to develop and stabilize its tertiary structure thus final folded model of proteins is necessary for their functional role (Kuhlman and Bradley, 2019). Similarly, molecular docking is commonly used as a scientific source in computer based drug designing to understand the unique and particular properties residing in different sequences of proteins (Saikia and Bordoloi, 2019). Investigating the non-covalent interaction of proteins and to predict their structural association with different sets of ligand help to explore the potential drug targets with functional and biological values which always serve as the foundational approach for drug design (De Vivo and Cavalli, 2017).

Rauwolfia serpentina (family *Apocynaceae*) is a well-known traditional medicine for the treatment of hypertensive and neurological disorders (Vivien, 1955; Lobay, 2015; Soni et al., 2016). In addition, this medicinal plant is reported for many pharmacological properties due to the presence of various secondary metabolites especially the variety of alkaloids belong to the indole alkaloid family, ajmaline, ajmalicine, reserpine, serpentine and others (Srivastava et al., 2006; Azmi and Qureshi, 2012a). Particularly in last decade, the role of methanolic roots extracts of *R. serpentina* was established in managing diabetic dyslipidemia by highlighting its hypoglycaemic, hypolipidaemic, antioxidant and haematinic potentials (Azmi and Qureshi, 2012b; Azmi and Qureshi, 2013). Beside this, *in silico* activity of the alkaloids of *R. serpentina* was investigated and confirmed their action as insulin receptor activators (Ganugapati et al., 2012) which was also validated by *in vivo* study conducted in fructose-induced type 2 diabetic mice model (Azmi and Qureshi, 2016). Another ligand-based study in 2013, screened indole alkoloids of *R. serpentina* against aldose reductase (AR) enzyme that involved in diabetic complications and found two structurally distinct plant-derived leads (indobine and indobinine) as potential inhibitors of this enzyme (Pathania et al., 2013). Later in 2016, transcriptome-wide identification of *R. serpentina* microRNAs was reported and predicted their potential targets as miRNA-mediated gene controlling biological processes in plant (Prakash et al., 2016). Therefore, this *in silico* study was designed to investigate the inhibitory potential of the alkaloids (ligands) of the roots of *R. serpentina* against HMGCR and to understand the structural basis of their interactions.

2. Materials and methods

2.1. Protein of interest

In beginning, the 3D structure of human HMGCR was retrieved from the Protein Data Bank (PDB) database. The selected structure of HMGCR has PDB-ID '1HW9', characteristically bound with Simvastatin, was used for investigation (Berman et al., 2000). This structure has been resolved through X-ray Diffraction at resolution 2.33 Å, with 0.248 as R-value Free and 0.222 as R-value Work.

2.2. Docking parameters

For the purpose of docking, Molegro Virtual Docker (MVD) software (Thomsen and Christensen, 2006) have been used. After proper refinement of three dimensional (3D) structure of protein (HMGCR, PDB ID: 1HW9), this PDB structure file was imported to the MVD work space. Imported protein was then prepared by assigning the missing bonds, hydrogen(s) and charge(s). The potential binding sites were considered and kept in focus in accordance with the reported structural mechanism for statin inhibition of HMGCR (Istvan and Deisenhofer, 2001) and the crystal structure of HMGCR (Istvan et al., 2000).

2.3. Ligands

After the thorough literature review, the structures of twelve (12) potential alkaloids were taken from the PubChem database as well as online open structural source and used as ligands in the present study mentioned in Table 1 (Pathania et al., 2015). All 12 ligands from these sources were first downloaded in mol2 format, then imported to the MVD workspace and finally optimized for docking studies.

2.4. Docking simulations

Once the protein and all the ligands prepared, docking simulations were started. Water molecules and cofactors were also included while performing the docking. The MVD docked each ligand into the binding site of HMGCR. During this study, ten docking runs were conducted and 10 docking solutions were obtained (poses) for each ligand and ranked them in order of increasing energies of the interaction. The pose with lowest energy was selected, along with those hydrogen interactions that were formed in between the ligand and amino acids present in the active site of the selected protein and were also analyzed for the top score poses. For predicting the binding pose of a ligand (drug candidate), MVD evaluate ligand conformations and estimate energy of interaction between each docked conformation and the protein by using the scoring function that was derived from PLP (Gehlhaar et al., 1995; Yang and Chen, 2004).

3. Results

The binding energies were obtained from the interactions of the selected alkaloids (Table 1). Out of twelve compounds, five of them including compound 1 (ajmalicine), 2 (reserpine), 3 (indobinine), 4 (yohimbine), and 5 (indobine) have secured the highest MolDock score (Table 2). The docking results indicated that the ajmalicine (compound 1) has highest score (-112.42 kcal/mol) and formed eight hydrogen bonds within the active site residues (V772 and G773, N771, T758 and K691) of HMGCR (Figs. 1 and 4). Whereas compound 2 (reserpine) with docking energy −108.103 kcal/mol showed the formation of about twelve hydrogen bonds with the amino acids such as V772, S774, N771, Y761, A769, A768, G773, T758 of HMGCR (Fig. 1). The third highest docking score (−99.47 03 kcal/mol) showed by compound 3 (indobinine) which interacted with the active site residue D690 and formed only one hydrogen bond (Figs. 1 and 4). The docking of compound 4 (yohimbine) generated score −98.8413 kcal/mol with four hydrogen bonds and the amino acids that participated in interactions were T758, S775 and G773 (Fig. 1). Compound 5 (indobine) with score −98.7336 kcal/mol formed two hydrogen bond with K843 and I756 (Figs. 2 and 4).

Beside this, the moderate docking scores were computed in compound 6 (neoajmalicine), which formed four hydrogen bonds and G773, K691, K692 and T689 were involved in interactions while compound 7 (serpentine) formed nine hydrogen bonds and amino acids including N697, G773, E801, T689, V772, K692, K691) were found interactive (Figs. 2 and 4).

Compound 8 (isoajmaline) formed five hydrogen bonds (K691, G773, K692, S774) and compound 9 (sarpagine) formed three hydrogen bonds (Figs. 2–4). Compound 10, 11 and 12 (papaverine,

Table 1
Structures of 12 Alkaloids from the roots of *R. serpentina*.

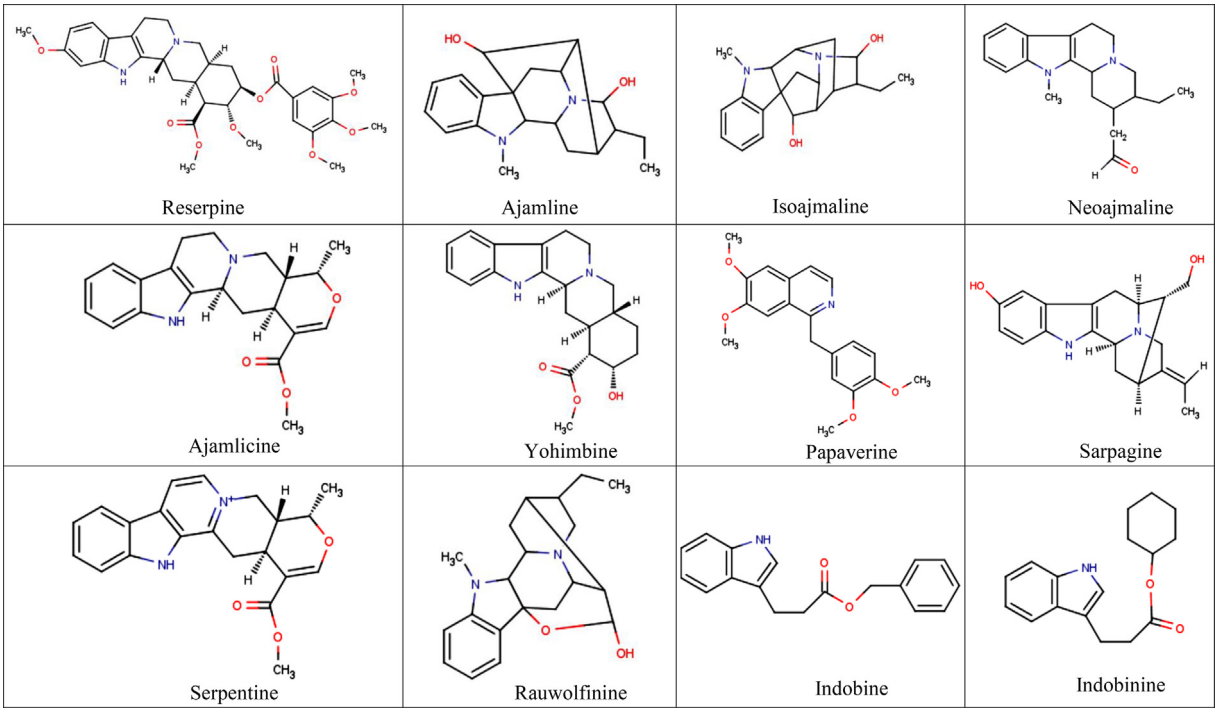

Table 2
MolDock scores and hydrogen bond interactions of docked compounds with HMGCR.

S. No.	Compound	Ligands	Mol Dock Score (kcal/mol)	Hydrogen Bond (kcal/mol)	Amino Acids Interaction
1	1	Ajmalicine	−112.42	−8.46202	Asp 690, Lys 635, Ser 684, Arg 590, Ala 751, Lys 692
2	2	Reserpine	−108.103	−8.9883	Asn 755, Lys 691, Lys 735, Ser 684, Arg 590, Lys692, Ala 751
3	3	Indobinine	−99.4703	−1.95566	Asp 690
4	4	Yohimbine	−98.8413	−7.5005	Asn 755, Asp 690, Arg 590, Ser 684, Lys 735, His 752, Lys 692
5	5	Indobine	−98.7336	−3.73768	Asn 755, Asp 690, His 752
6	6	Neoajmaline	−97.6681	−5.18324	Lys 691, Arg 590, Asp 690, Lys 692, Ala 751
7	7	Serpentine	−97.445	−4.26799	Asn 755, Arg 590, Ser 684, Lys 735, Lys 692, His 752
8	8	Isoajmaline	−92.9784	−3.9796	Glu 559, Asn 755, Ser 565
9	9	Sarpagine	−92.1083	−2.63783	Glu 559, Ser 684, Lys 735
10	10	Papaverine	−89.0635	−5.03754	Lys 735, Ser 684, Asn 755, Lys 691, Ser 565
11	11	Rauwolfinine	−79.1413	−2.7687	Glu 559, Ser 565
12	12	Ajmaline	−78.4868	−5.23382	Ser 684, Arg 590, Lys 692

rauwolfinine and ajmaline) were seen to interact with the active site residues by forming five, two and three hydrogen bonds respectively (Table 2 and Figs. 3 and 4).

4. Discussion

Molecular docking is a computational approach used to determine the interaction of a protein with the ligand through forming a supramolecular complex or assembly which may enhance or inhibit the biological function (De Vivo and Cavalli, 2017; Saikia and Bordoloi, 2019). The key of drug designing is based on the binding alignment of small molecules (either inhibitor or activator) with protein targets and on the basis of correct structural conformation held together through non-covalent interaction of ligand within the active site of the protein (Du et al., 2016). Therefore, in this effort, the molecular association of different alkaloids of *R. serpentina* with HMGCR was screened to predict their inhibitory impact on this enzyme.

In order to understand and explore the binding sites interaction of alkaloids with target protein (HMGCR), the final product of molecular docking was clustered to specify the ligand-binding free energy and optimal docking energy conformation and termed as best docked structure. In this regard, five tested alkaloids ajmalicine, reserpine, indobinine, yohimbine and indobine (compound 1, 2, 3, 4, and 5) showed highest MolDock score in the present study (Table 2).

Formation of hydrogen bonds primarily represents the non-covalent interaction between a positive charged hydrogen-atom and an electronegative atom (oxygen or nitrogen) with lone pairs of electrons (Yunta, 2017). In present findings, compound 1, 2, and 7 (ajmalicine, reserpine and serpentine) formed highest number of hydrogen bond during their interaction with HMGCR (Figs. 1, 2 and 4). This is due to the ionic strength of hydrogen bonds in protein, most of the hydrogen bonds occur in networks, in which each donor atom contributes in multiple interactions with acceptors and every acceptor interacts with many donors (Chen et al., 2016; Yunta, 2017). Therefore, molecular hydrogen-bonding potentials (MHBPs) are considered as practical computational approach in determining the structural activity relationship of drug designing (Chen et al., 2016; Caron and Ermondi, 2017).

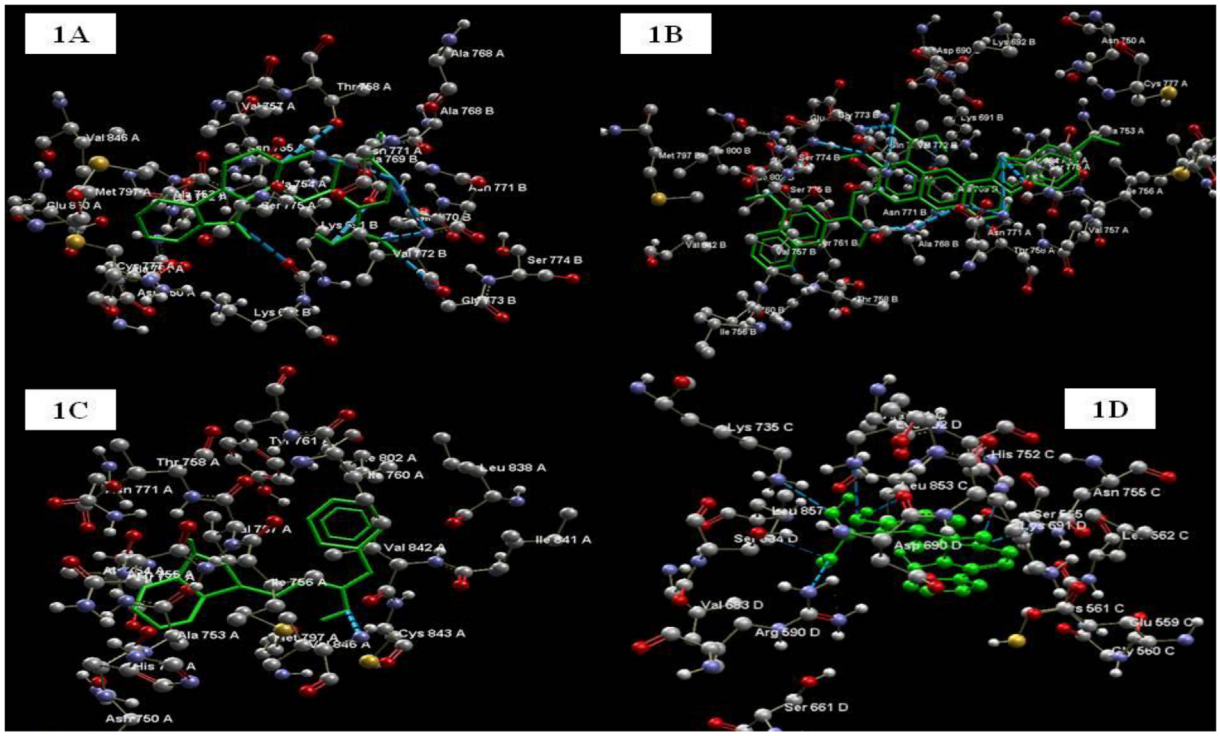

Fig. 1. Docking of ligands (1A) Ajmalicine, (1B) Reserpine, (1C) Indobinine and (1D) Yohimbine within the active site of HMGCR (ligands are presented in green color and hydrogen bonding with blue dashed line).

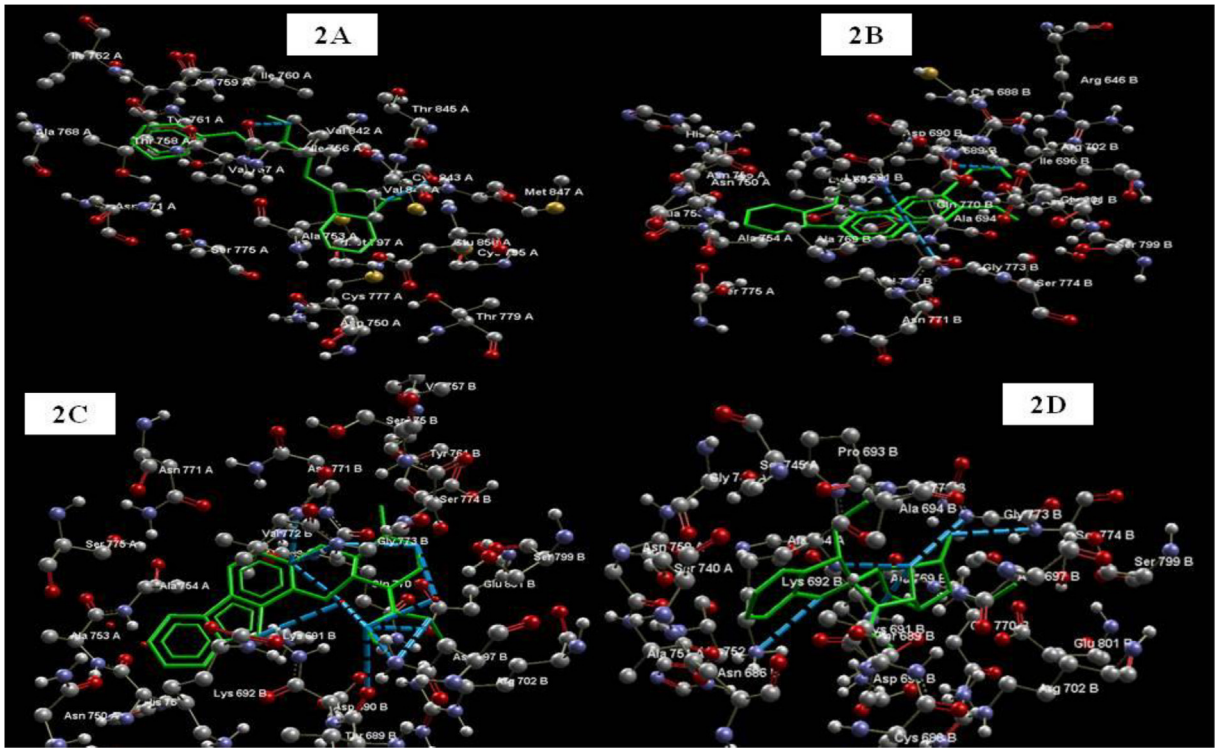

Fig. 2. Docking of ligands (2A) Indobine, (2B) Neoajmalicine, (2C) Serpentine and (2D) Isoajmaline within the active site of HMGCR (ligands are presented in green color and hydrogen bonding with blue dashed line).

It was also reported that establishment of hydrogen bonds contributes in protein structural stability, as far this pattern accounts for extensive hydrogen bonding in α-helices and β-pleated sheets (Chen et al., 2016; Du et al., 2016; Zhou and Pang, 2018). Moreover, stability in protein structure is actually the representation of the difference in free energy between the folded structural state and the unfolded structural state (Perez et al., 2016). In addition to this, compound 8 and 10 also

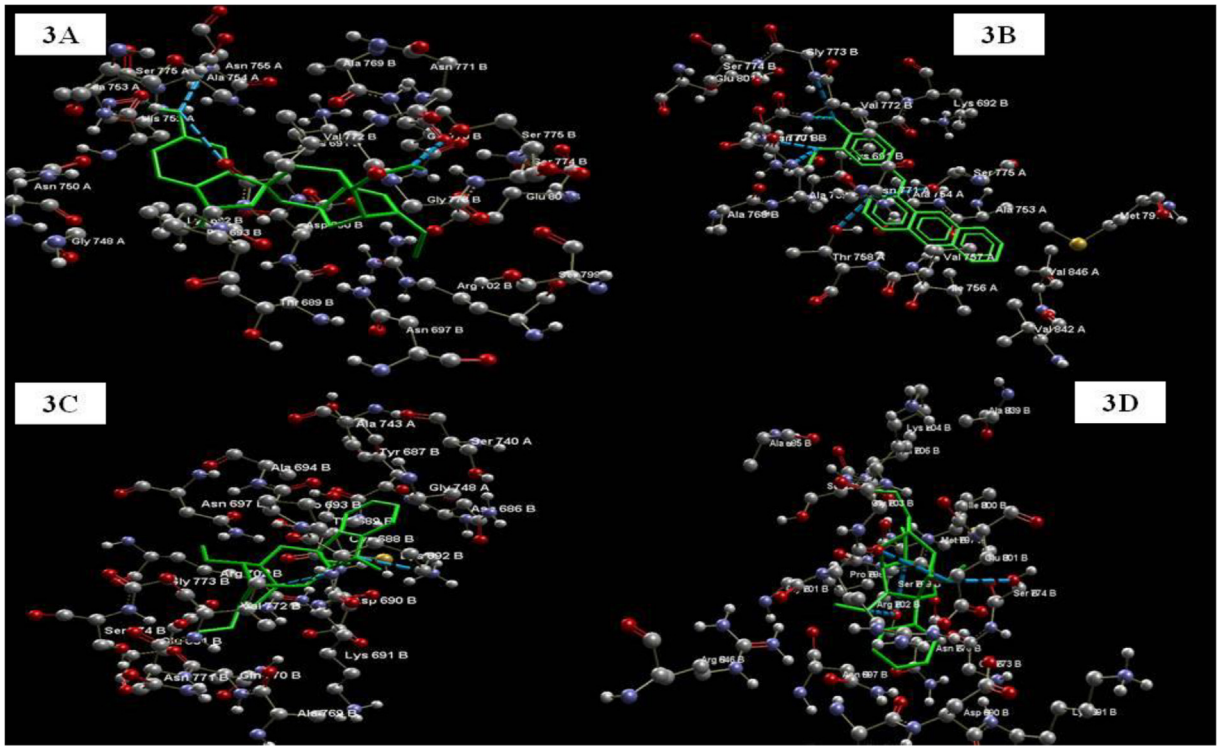

Fig. 3. Docking of ligands (3A) Sarpagine, (3B) Papaverine, (3C) Rauwolfinine and (2D) Ajmaline within the active site of HMGCR (ligands are presented in green color and hydrogen bonding with blue dashed line).

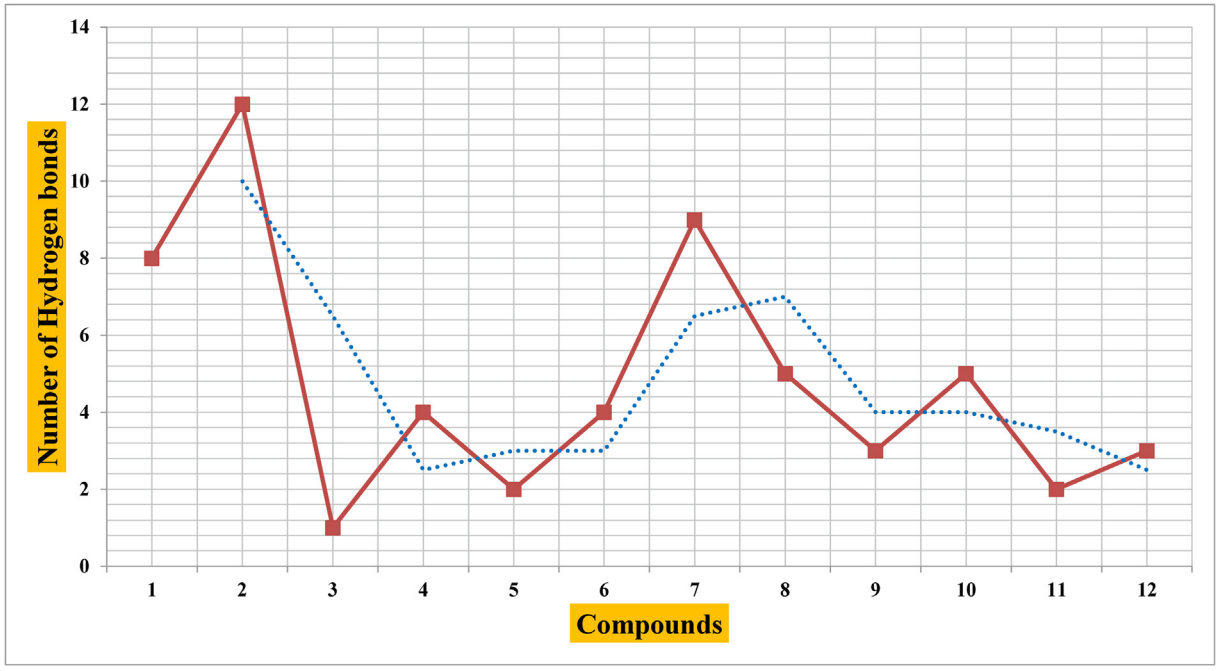

Fig. 4. MHBP of docked alkaloid compounds with HMGCR (Red line indicates the binding frequency and blue dotted line indicates the moving average trendline with 2 hydrogen bonds per average with each compound).

exhibited good number of hydrogen bonds with HMGCR (Fig. 4) and this may be characterized as one of the strengthening attribute of this work which contributes in the stability of protein-ligand interaction. Similarly, in virtual format of screening, each hydrogen donor and acceptor in a protein are observed to establish an interaction within the folded protein structure or to the external solvent molecules (Chen et al., 2016; Caron and Ermondi, 2017; Zhou and Pang, 2018).

Along with hydrogen bonding, some hydrophobic interactions were also observed in between docked ligand and active site residues including H752, L853 and L857 specifically at H752 residue with compound 4 (Yohimbine), 5 (Indobine) and 7 (Serpentine).

It has also been reported that a class of statins like compactin, fluvastatin, and others (PDB ID: 1HW8, 1HW1, 2Q1L) inhibits the activity of HMGCR in the similar way (Istvan and Deisenhofer, 2001). According to Istvan and Deisenhofer in 2001, the binding pocket for HMGCR resides or located at amino acid positioned between 682–694 residues and generally referred as '*cis-loop*'. Moreover, another study reported the crystal structure of the catalytic portion of human HMGCR, this *cis-loop* was recognized as the most significant structural residues present in the binding pocket of this rate-limiting enzyme (Istvan et al., 2000). Similarly, the present top five alkaloids with highest MolDock scores showed significant interactions within and between *cis-loop* residues of HMGCR. Further, the preferred interactions of ligands (alkaloids) with D690, K691, and K692 positions were also observed with prominent residues position of HMGCR (Table 2). These prominent residues including D690, K691, and K692 are important due to the location of *cis*-peptide bond between C688 and T689, which established the future importance of any kind of competitive as well as non-covalent interaction with inhibitors like statin (Istvan et al., 2000).

In present work, the third important focus was to explore any further interaction of ligands with residues at E559 and D767. The main reason for finding this pattern of alkaloidal interaction with these residues was because of their spatial positions in front of the active site of HMGCR (Istvan et al., 2000). Interestingly, isoajmaline and rauwolfinine which already have had no interaction with any residue located in *cis-loop* position of HMGCR, but they showed amino acid interaction at E559 position, while rest of alkaloids didn't show any interaction at E559 residue of HMGCR (Table 2). In 2012, a study reported the glucose lowering activity of low doses of methanolic roots extract of *R. serepntina* might be due to presence of alkaloids as potential chemical compounds in extract (Azmi and Qureshi, 2012b). The given possibility in 2012 becomes fortified from this present *in silico* activity that these alkaloids can inhibit the HMGCR's role in body and improve the circulation of glycemic status.

Interestingly, another *in vivo* study in 2016 reported the role of methanolic roots extract of *R. serepntina* in suppressing the activity of HMGCR in type 2 diabetic mice (Azmi and Qureshi, 2016). Earlier to this, the same group also claimed the significance of the same extract in improving the glycaemic and antiatherogenic indices in diabetic groups of mice (Azmi and Qureshi, 2012b). On the basis of these pre-clinical findings and the present *in silico* outcomes have been proved that the therapeutic effect of *R. serepntina* in lowering hyperglycaemia, hypercholesterolemia and managing the atherogenic dyslipidaemia (Azmi et al., 2015; Azmi et al., 2018) unquestionably based on the presence of alkaloids potentially ajmalicine, reserpine, indobinine, yohimbine and indobine which inhibit HMGCR and indirectly produced the therapeutic effect of roots extract in type 1 and type 2 diabetic mice models.

5. Conclusion

This work concludes that five alkaloids from the roots of *R. serepntina* including ajmalicine, reserpine, indobinine, yohimbine and indobine showed best fit interaction with active site of HMGCR and are predicted to be the most potent inhibitors of HMGCR by exhibiting the highest docking scores.

6. Future implications

The predicted five alkaloids of *R. serepntina* (ajmalicine, reserpine, indobinine, yohimbine, and indobine) may serve as potential therapeutic leads for the development of clinically effective HMGCR inhibitors. Further, lead optimization and preclinical and clinical investigations on these compounds are necessary to develop potential drug entity for the treatment of hypercholesterolemia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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