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Molecular docking studies of B-RAF expression inhibitors identified from *Strychnos potatorum* (Thethankottai)

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ABSTRACT

The B-Raf mutation has been of a potential therapeutic relevance in melanoma and other cancersin the cancer research community. After the primary set back with the results achieved in clinical trials with the first generation RAF proto-oncogene serine/threonine-protein kinase (RAF kinase) inhibitor, Sorafenib, the validation of B-Raf as a therapeutic target kinase inhibitors is very promising. Discovery Studio 4.0 provides a set of protocols for predicting and analyzing the interaction between protein and ligands. Docking experiments were carried out for compounds identified from Strychnos potatorum seed extract with B-Rafkinase (3C4C) using Accelry's Discovery Studio 4.0. Out of the five compounds that were selected from Strychnos potatorum seed, four compounds docked with B-Raf kinase,of which, NSC606748 and Strychnine that showed best interactions with 3C4C can be considered as lead compounds for cancer therapeutics.

Keywords: B-Raf mutation, Rapidly Accelerated Fibrosarcoma (RAF) cancer therapeutics, *Strychnos potatorum* seed, Molecular docking.

INTRODUCTION

B-Raf mutations are found in a wide range of cancers.There is a development towards the occurrence of mutations in cancer types in which a substantial proportion of cases are known to harbor RAS mutations (for example, malignant melanoma, borderline ovarian cancers etc.). The apparent association between the presence of B-Raf and RAS mutations in similar cancer types suggests that activation of the RAS–RAF–mitogen-activated protein (MAP)-kinase (MEK)–extracellular signal-regulated kinase (ERK)–MAP kinase pathway can be achieved by mutation at various levels in the pathway and that the pathway is activated in a substantial proportion of cases in these cancer types.Mutation occurs most frequentlyin B-Raf oncogenic protein kinase. Furthermore, inhibitors targeting "active" protein kinases have demonstrated significant utility in the therapeutic repertoire against cancer. By using a structure-guided drug discovery approach, aneffective and selective inhibitor of B-Raf kinasecan be discovered[1-3]. *Strychnos potatorum* (Loganiaceae**)**is a semi-erect diffuse under shrub, distributed throughout India. The extract from the leaf, root, stem and callus of *Strychnos potatorum* showed anti-fungal and anti-cancer property. Literature survey revealed the extract of this plant has been showed to exert anti-diabetic, anti-ulcer, anti-diarrhoeal and anti-cancer effect[4-9].

The seeds of *Strychnos potatorum* is used from ancient times in traditional medicines. The seed consists ofalkaloids, flavonoids, saponins, sterols, phenols, lignins, glycosides and tannins. In Ayurveda it is used for conditions such as; hepatopathy, gastropathy, bronchitis, chronic diarrhea, renal and vesicle calculi, leucorrhea, diabetes, etc. Presence of alkaloids, flavonoids, glycosides, lignin, phenols, saponins, sterols & tannins is eminent from literature survey. The data available from various experiments in the literature indicates strongly the usage of this plant as a potent therapeutic agent for treating various ailments[10,11].

In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is universally used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the ligands. Hence, docking plays a significant role in the rational drug design.

Molecular docking studies indicate how two or more molecular structures interact with each other for example, drug and enzyme or receptor of protein, fit together. Molecular docking software's are mainly used in drug research. The most important application of docking software is virtual screening.Virtual screening selects the most interesting and promising molecules from an existing database for advanced research. This places demands on the used computational method which must be fast and reliable[12-14].

- Kingdom: Plantae
- Class: Angiosperms
- Subclass: Eudicots
- Superorder: Asterids
- Order: Gentianales
- Family: Loganiaceae
- Genus: Strychnos

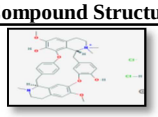
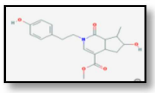
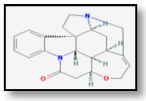
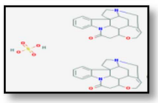
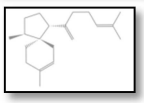
Fig.1. Strychnos potatorum seed

MATERIALS AND METHODS

Compounds identified from *Strychnos potatorum* seed

The compounds, Tubocurarine chloride, NSC606748, Strychnine, Strychnine Sulfate, Viscida-4,11(18),14-triene present in the seeds of *Strychnos potatorum* were taken from PubChem database, and were used for binding analysis with B-Raf kinase (current therapeutic target for cancer) (Table 1).

Table1. Five Compounds Identified inthe seed of *Strychnos potatorum*

S.No	Compound Name	Compound Structure	Molecular Formula	Molecular Weight
1	Tubocurarine chloride		$C_{37}H_{42}Cl_2N_2O_6$	681.645180 g/mol
2	NSC606748		$C_{19}H_{23}NO_5$	345.389620 g/mol
3	Strychnine		$C_{21}H_{22}N_2O_2$	334.411580g/mol
4	Strychnine sulphate		$C_{42}H_{46}N_4O_8S$	766.901640 g/mol
5	Viscida-4,11(18),14-triene (**lipid**)		$C_{20}H_{32}$	272.4681 g/mol

Ligand preparation

The compounds in this study were collected from published research articles in recentyears. The three dimensional structure of compounds taken for binding analysis was downloaded in .sdf format from PubChem. Hydrogen bonds were added. Lipinski properties such as Molecular weight, AlogP98, number of hydrogen bond donors and acceptors, Blood Brain Barrier (BBB), CYP2D6for the compounds were obtained while assessing ADMET properties (Table 2).

Protein preparation

The PDB is a key resource in areas of structural biology, a key repository for 3D structure data of large molecules. The molecule taken is 'B-Raf kinase' with PDB ID - 3C4C, resolution factor of 1.60Å and the method of incorporation is X-ray diffraction method. The ligand and crystallographic water molecules were removed from the protein and the Chemistry of the protein was corrected for missing hydrogen. Unfilled valence atoms were connected using alternate conformations and valence monitor options. Following the steps of preparation, the protein was submitted to energy minimization using the CHARMm Force field.

Docking studies

The docking method used in this study is Flexible docking. The five compounds that are screened by Ligand-Fit is docked with the modeled protein. A protocol called "Dock-ligands" (Ligand-Fit) is selected among those listed under receptor-ligand interaction protocol cluster. Each ligand compoundis given as input in the parameter meant for "input ligands" and the protocol was run.The Dockscore of the best poses docked in to the B-Raf kinase for all the 5 compounds is calculated.Theprotein-ligand interaction is followed byfinal energy refinement of the ligand pose with the active site residues.

RESULTS AND DISCUSSION

The retrieved crystal structure from PDB ofB-Raf kinase(PDB ID 3C4C) have structural weight as 64579.25. This protein is homo-dimer with two chains namely 'A' and 'B. Each chain has 280 amino acid residues. Out of five different compounds (ligands) taken for docking analyse,only four compounds docked with the protein B-Raf kinase, out of which one compound showed the best interaction with the active site receptors. Docked pose of the compound with protein (B-Raf kinase) is presented in Figure2.The five compounds(B-Raf kinase) with ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties and the molecules' Heat map is presented in Figure3. The chart showing Residue and five compounds'Favorable, Unfavorable,Hydrogen bond and Hydrophobic interactions is represented in Figure 4.Thedock-score values include CDOCKER Energy, Lib-Dock score, VDW (van der Waals)Energy,Binding energy, Ligscore 1&2, Piecewise Linear Potential (PLP) - PLP1 and PLP 2, Jain, Potential of Mean Force- PMF,Hydrogen Bond Total obtained using the LigandFitprotocol of Discovery Studio 4.0[14].

The dock score values for the various compounds (ligands) identified from *Strychnos potatorum* seed with B-Raf kinase are shown in Table 2. Ligands NSC606748 showed highest dockscore with B-Raf kinase when compared to other ligands. Ligands Strychnine form six hydrogen bonds, Strychnine sulphate form four hydrogen bonds, while Viscida-4,11(18),14-triene forms zero hydrogen bonds with the target protein. The detailed information about the atoms involved in forming the hydrogen bond and the number of hydrogen bonds formed between the ligands and enzyme are provided in Table 3.

To ensure that the ligand orientation obtained from the docking studies was likely to represent valid and reasonable binding modes of the inhibitors, the ligand Fit program docking parameters had to be first validated for the crystal structure's active site. Protein utilities and health protocol of Discovery Studio was used to find out if the active site contains amino acids such as Ile 463,Ala 481,Lys 483,Leu 505,Leu 514,Ile 527,Thr 529,Gln 530,Trp 531,Cys 532,Phe 583,Asp 594,Phe 595. Results of docking showed that the compounds binds to the active site residues which indicates that these compounds can inhibit the B-Raf kinase. Further clinical trials are required to validate these compounds present in the seeds of *Strychnos potatorum.*

Table2. Physiochemical Properties of Compounds Identified in *Strychnos potatorum*

S.No	Compound name	Molecular Weight	AlogP98	H-Bond DONOR	H-Bond ACCEPTOR	BBB	CYP2D6
1	Tubocurarine chloride	681.645	3.851	3	8	-0.238	-2.2849
2	NSC606748	345.39	1.264	2	5	-1.164	-4.44866
3	Strychnine	334.412	0.109	0	3	-0.639	-3.35069
4	Strychnine sulphate	766.902	1.145	2	10	-0.639	-3.35069
5	Viscida-4,11(18),14-triene	272.468	6.579	0	0	1.88	0.124875

Table3. Results for Protein-Ligand Interaction

S. No	Compound name	CDOCKER Energy	LibDock score	VDW Energy	Binding energy	Lig score 1	Lig score 2	-PLP1	-PLP 2	Jain	-PMF
1	Tubocurarine chloride	-	-	-	-	-	-	-	-	-	-
2	**NSC606748**	3.362	**100.53**	-100.028	-46.715	3.1	5.06	63.33	63.3	0.56	90.2
3	Strychnine	-102.884	89.597	-95.311	75.078	2.07	4.04	90.06	90.77	6.71	70.86
4	Strychnine sulphate	-77.957	82.827	-92.825	-31.018	1.38	3.95	80.37	80.02	5.39	49.62
5	Viscida-4,11(18),14-triene	-57.674	84.264	-96.64	-39.052	1.17	3.87	69.13	67.76	5.95	82.88

Table4. Statistical Residue Analysis

Statistical Residue Analysis

Total Interaction Count

Favorable	Unfavorable	HydrogenBond	Charge	Hydrophobic	Halogen	Other
3544	147	1240	25	2545	0	58

Top 5 Residues with Favorable Interactions (5)

Residue	Favorable	Unfavorable	HydrogenBond	Charge	Hydrophobic	Halogen	Other
A:CYS532	369	5	146	0	319	0	21
A:ILE463	310	46	63	0	296	0	0
A:VAL471	288	0	0	0	288	0	0
A:ARG603	250	16	118	3	186	0	0
A:ALA481	249	1	0	0	249	0	0

Top 5 Residues with Unfavorable Interactions (5)

Residue	Unfavorable	Favorable	HydrogenBond	Charge	Hydrophobic	Halogen	Other
A:ILE463	46	310	63	0	296	0	0
A:TRP531	22	230	25	3	202	0	7
A:ARG603	16	250	118	3	186	0	0
A:PHE583	13	229	6	8	222	0	5
A:LEU514	7	133	0	0	133	0	0

Top 5 Residues with HydrogenBond Interactions (5)

Residue	HydrogenBond	Unfavorable	Favorable	Charge	Hydrophobic	Halogen	Other
A:CYS532	146	5	369	0	319	0	21
A:GLU533	131	0	131	0	0	0	0
A:ARG603	118	16	250	3	186	0	0
A:SER535	118	0	134	0	31	0	0
A:GLY534	110	1	155	0	48	0	0

Top 5 Residues with Charge Interactions (5)

Residue	Charge	Unfavorable	HydrogenBond	Favorable	Hydrophobic	Halogen	Other
A:LYS473	10	2	16	195	184	0	0
A:PHE583	8	13	6	229	222	0	5
A:ARG603	3	16	118	250	186	0	0
A:TRP531	3	22	25	230	202	0	7
A:LYS475	1	3	1	63	62	0	0

Top 5 Residues with Hydrophobic Interactions (5)

Residue	Hydrophobic	Unfavorable	HydrogenBond	Charge	Favorable	Halogen	Other
A:CYS532	319	5	146	0	369	0	21
A:ILE463	296	46	63	0	310	0	0
A:VAL471	288	0	0	0	288	0	0
A:ALA481	249	1	0	0	249	0	0
A:PHE583	222	13	6	8	229	0	5

Top 5 Residues with Other Interactions (4)

Residue	Other	Unfavorable	HydrogenBond	Charge	Hydrophobic	Halogen	Favorable
A:TRP604	25	4	35	0	60	0	98
A:CYS532	21	5	146	0	319	0	369
A:TRP531	7	22	25	3	202	0	230
A:PHE583	5	13	6	8	222	0	229

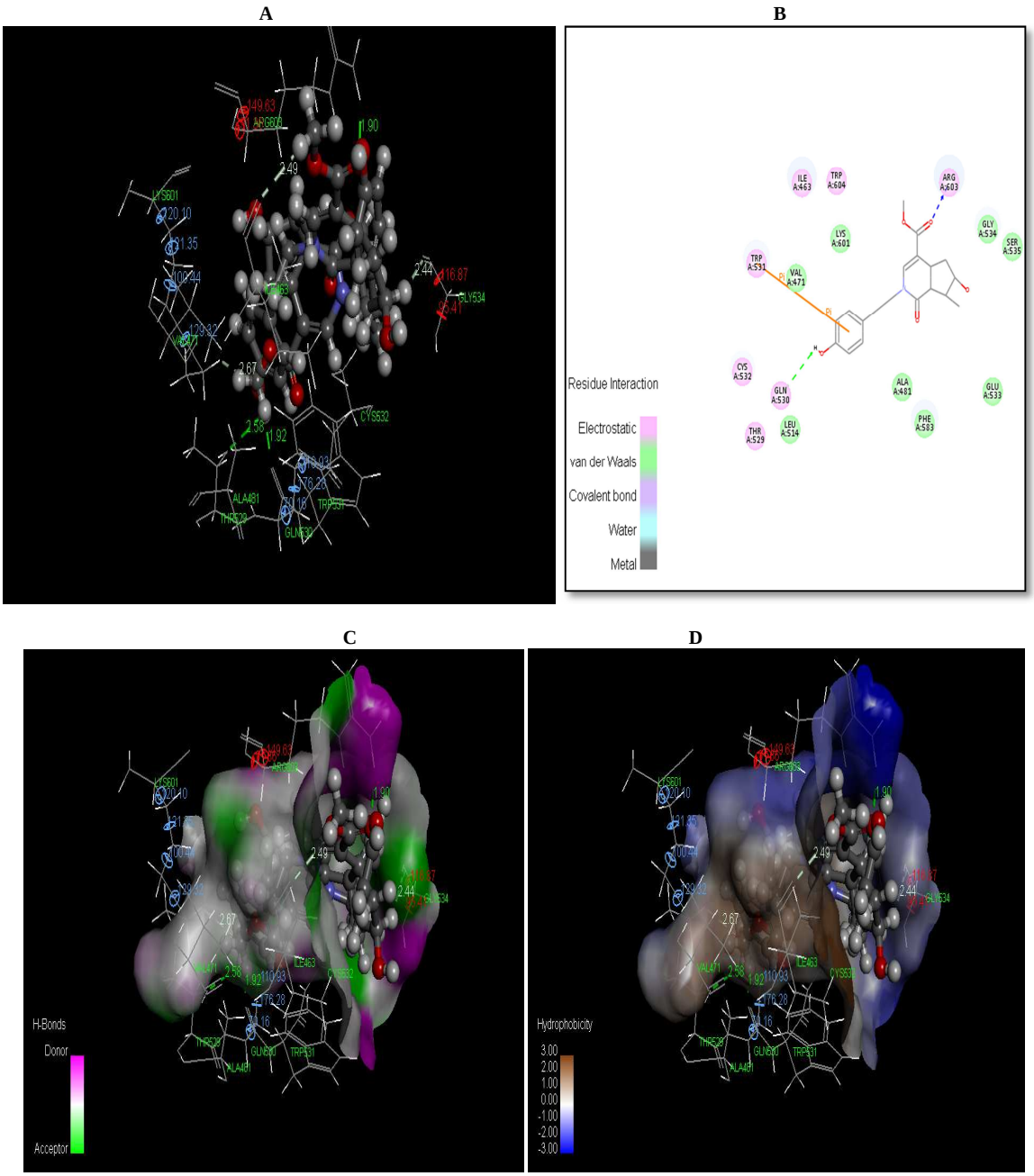


Fig.2. Summary of docked pose of compound(NSC606748)with B-Rafkinase. Docked model of (A) NSC606748 Ligand interaction, (B) 2D Diagram, (C) H-Bonds, (D) Hydrophobicity with B-Raf kinase

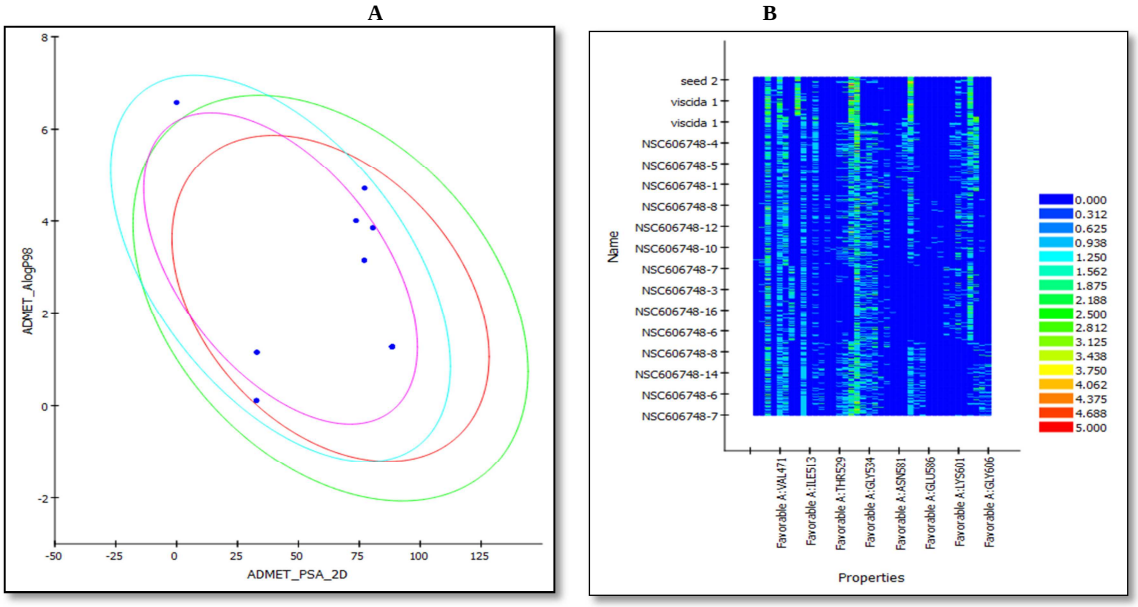


Fig.3. Summary of Five compounds with B-Raf kinase with ADMET and Molecule Heat map. Docked five compounds of (A) ADMET (B) Molecule Heat Map

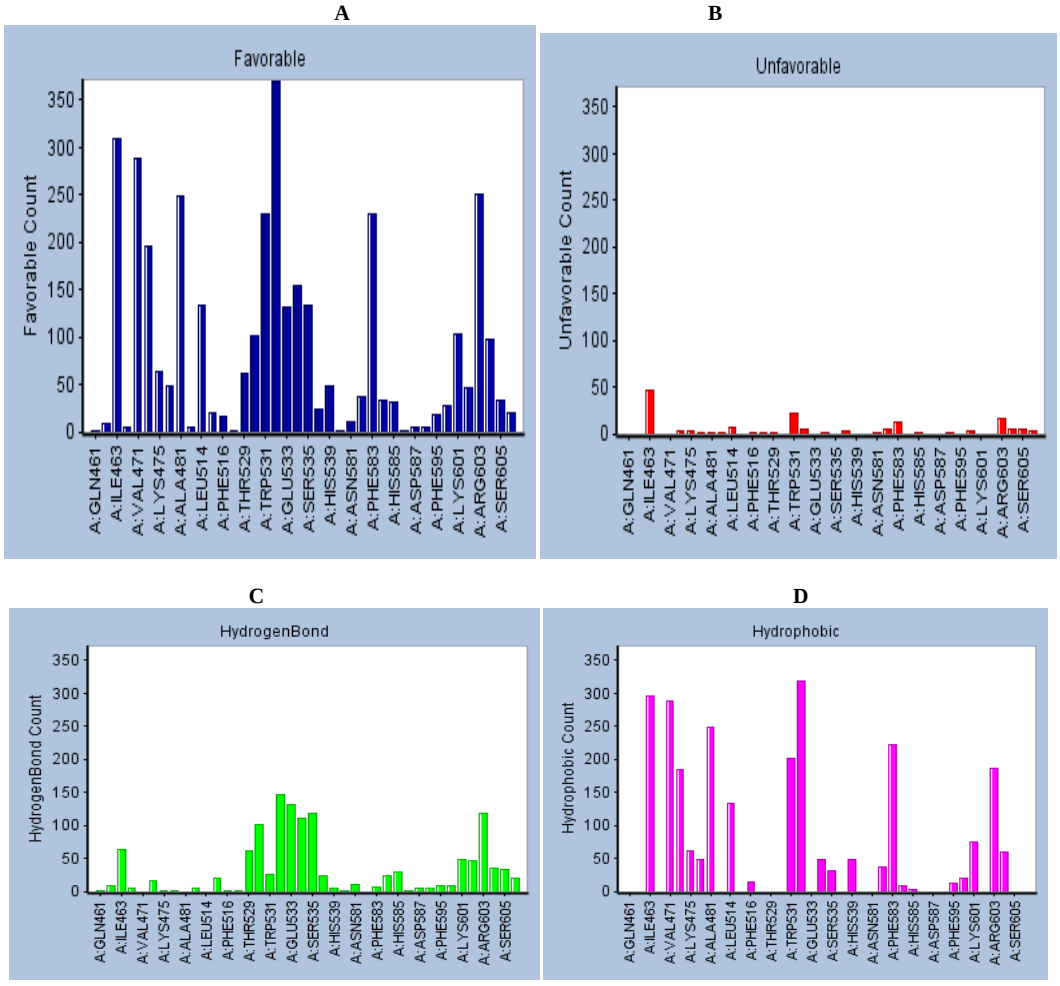


Fig.4. Summary of Five compounds of Residue Interaction Histograms. Docked Residue Interactions (A) Favorable, (B) Unfavorable, (C) Hydrogen Bond, (D) Hydrophobic

CONCLUSION

Bioinformatics approach contribute supportive evidences for the promising action of a drug molecule under research and also help in saving time and minimizing the number of pre-clinical trials. It iscrucial that Bioinformatics and pharmaceutics complement each other and play an equal role in drug research which will prove effective in developing novel, specific and safe drugs to achieve a continual response. The present study indicates that the bioactive compounds from *Strychnos potatorum* seeds which shows a strong binding affinity towards B-RAF kinase thus inhibiting its overexpression, and therefore can be used in the treatment of cancer. This study brings a focus towards this plant that, when administered during the treatment of Cancer may block B-Raf kinase expression.

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Conflict of Interest
The authors declare that there is no conflict of interests regarding the publication of this paper. Also, they declare that this paper or part of it has not been published elsewhere.

Contribution of the Authors
1. R.P. Sasikala made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data;
2. R.P. Sasikala, P.G. Stella and Dr. K.S. Meena participated in revising it critically for important intellectual content; and
3. Dr. K.S. Meena gave approval of the final version of the manuscript to be submitted for publication.

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