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Research Article

RBP-J AS A THERAPEUTIC TARGET TO RHEUMATOID ARTHRITIS- AN *IN SILICO* STUDY

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ABSTRACT

Use of herbal medicines among patients under Rheumatoid Arthritis (RA) is widespread. However they lack drug targeted therapy and synthetic drugs have one or the other side effects. In this paper, we have reviewed the literature to determine the possible interactions between plant isolates and synthetic drugs. *Kirganelia reticulata* (Poir.), traditionally used to treat rheumatism was searched for its bioactive compounds published. There were quiet several compounds identified. Among them Scopoletin, Methyl brevifolin, Methyl gallate, Ellagic acid, Kaempferol and Quercetin followed Lipinski's rule of 5. Naproxen, which is an available NSAID was used as a standard drug. RBP-J is a protein involved in both molecular pathways Notch and Toll-like receptor, was selected as a therapeutic target to RA. Based on binding energy (-7.65) and number of hydrogen bonds (7) formed in the docking process Ellagic acid was considered to be efficient in comparison with Naproxen. Interaction between herbal medicine and synthetic drugs is a potentially important safety issue. Patients taking NSAID's, DMARD's are at the highest risk.

KEYWORDS: Rheumatoid Arthritis, *Kirganelia reticulata*, RBP-J, Docking

INTRODUCTION

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic disease that affects the joints, often those in a person's wrists, fingers, and feet. Systemic inflammatory disorder that affect many tissues and organs, but principally attacks the joints producing an inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints. Although its cause is unknown, autoimmunity plays a pivotal role in its chronicityand progression. Analgesicand anti-inflammatory drugs, including steroids, are used to suppress the symptoms,while disease-modifying antirheumatic drugs (DMARDs) are often required to inhibit or halt the underlying immune process and prevent long-term damage. The availability of highly effective biologic therapies, recognition that early, intensive therapy and tight control of disease activity are important strategies, provides the opportunity to aim for clinical and radiographic remission in RA [1].

Therapeutic target:

Recombining binding protein suppressor of hairless is a protein that in humans is encoded by the *RBPJ* gene. RBP-J involved in both molecular pathways Notch and Toll-like receptor, is linked and manipulating that protein, could serve as a treatment for RA. Notch pathway is important in development and that the Toll-like receptor pathways are important in acute inflammation, and hence two things are linked in acute inflammation and cytokine production. Hence, the interest in these receptors involved in cell–cell communication has spilled over into multiple clinical disciplines and has paved the way to novel insights, ranging from Alzheimer's disease to cancer. Specific inhibitors that selectively target the activation of the Notch-signalling pathway are now available and enter the stage of clinical trials. The Notch receptor family includes four members in mammalians that are all anchored in the cell membranes as heterodimers and are involved in short-range cell–cell communication, cell-fate decision, patterning and cell polarity [2]. Proteolytic cleavage of the receptor releases the intracellular domains, which are targeted to the cell nucleus and associate with other transcriptional regulators, most importantly Rbpj [3]. Early investigations have showed that with the absence of RBP-J, the production of interleukin-6 and activation of certain Notch target genes decreases. These components bear also great potential for the treatment of other diseases, given that experimental data link this signalling pathway to diverse inflammatory and arthritic diseases.

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Herbal Medicines

Ayurveda literally means 'Science of Life', and is considered to be the traditional medicine of India. The origin of this system goes back to a far past, in which philosophy and medicine were not separated. Therefore, philosophical views have strongly influenced the Ayurvedic way of thinking. In contrast to Western science, where objective observation played a central role, the premise in Ayurveda was subjectivity: cognition of an object or phenomenon is established by individual perception [4]. In the last decades the use of herbal preparations has been growing in Western countries where exotic drugs are gaining attention. One of the reasons for the increasing interest in herbal medicines is the belief that, being natural and traditionally used, they are hence safe and harmless [5]. *Kirganelia reticulata* (Poir.) is a straggling shrub, belong to family Euphorbiaceae. The plant parts are being usually used in treatment of rheumatism at traditional practices, commonly called as Krishna nelli. Review of phytochemical investigations resulted in the isolation of tannic acid, friedelin, epifriedelinol, betulin, taraxerone, beta- sitosterol, glochidonol, octacosanol, taraxeryl acetate and 21-alpha-hydroxyfriedelan-3-one [6]. The preliminary antimicrobial and cytotoxicity activities of the organic extractives and the isolation of a coumarin, scopoletin from the chloroform soluble material of the methanol extract are reported [7]. Eight compounds (β-Sitosterol-3-*O*- β glucoside, stigmasterol-3-*O*- β-glucoside, methyl gallate, ellagic acid, corilagin, methyl brevifolincarboxylate, kaempferol and astragalin), including two flavonoid glycosides (rutin (quercetin 3-rutinoside) and quercetin 3-*O*- β –D-glucopyranoside (isoquercitrin)), were isolated from the butanol-soluble fraction of the methanolic extract of the leaves of *Phyllanthus reticulatus* by conventional methods [8]. Three compounds were isolated and identified as lupeol acetate, stigmasterol and lupeol from leaves of this plant by Jamal *et al*., (2008) [9].

MATERIALS AND METHODS

Proteomics of RBP-J

RBP-J: Recombination Binding Protein suppressor of hairless (B7Z8D8_HUMAN) was selected as target. The documentation entries describing protein domains, families and functional sites as well as associated patterns and profiles were identified using Prosite. Protein family, other physicochemical parameters and secondary structures were predicted using PSSM, Pfam, PROTPARAM and JPred respectively. The prediction of signal peptides and their cleavage sites, subcellular localization was done using SignalP and P-SORT. The three dimensional structure of target protein was taken from PDB (Protein data Bank) structural database.

Selection of Ligands

The above mentioned compounds were checked for the Lipinski's rule of 5 in PubChem (NCBI). The obtained ligands were analyzed for molecular formula, molecular weight, Log P, H bond donor, H bond acceptor and structure. Ligands namely Scopoletin, Methyl brevifolin, Methyl gallate, Ellagic acid, Kaempferol and Quercetin were further continued with docking studies. Naproxen was taken as standard drug and comparative study was done simultaneously.

Docking studies

Structure of RBP-J having keyword 3BRG was downloaded and hetero atoms were removed. This file was then opened in SPDB viewer to add the C-terminal Oxygen atoms. The active pockets on target protein molecule were found out using CASTp (Computed Atlas of Surface Topography of proteins) server. The ligands were drawn using Chem Draw Ultra 6.0 and assigned with proper 2D orientation (ChemOffice package). Energy of the molecules was minimized using Dundee PRODRG2 server. Autodock V3.0 was used to perform Automated Molecular Docking.

RESULTS AND DISCUSSION

UniProtKB/TrEMBL B7Z8D8 is a Recombining binding protein suppressor of hairless isolated from *Homo sapiens*, having 379 AA and evidence found to be at transcript level. The protein has two domains LAG1-DNAbind and Beta-trefoil. The position specific scoring matrix and HMM logo of respective domains along with its Prosite documentation entries and secondary structures are shown in fig 1. Its physicochemical parameters like molecular weight, pI, amino acid and atoms composition, extinction co-efficients are predicted using PROTPARAM and are shown in the fig 2. Scalar parameters give an idea for protein analysis and isolation procedure. Signal P 3.0 server predicts the presence and location of signal peptide cleavage sites in amino acid sequences. This method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks and hidden Markov models. Since the *C*, *S*, *Y* scores are less and based on probability regions of cleavage sites it is considered to be a non-secretory protein. The results of which are shown in fig 3. Using Psort the subcellular localization is found to be at a distance of 19.0 from nucleus and 12.0 from cytoplasm. Review of biological properties of protein is essential for extraction; however, is still a considerable challenge in proteomics. The idea of the comprehensive study of the proteome is more an idealism than a reality [10].

Fig 1: (A) PSSM of Beta-trefoil domain, (B) HMM logo of LAG1-DNAbind domain, (C) Documentation entries of RBP-J in prosite, (D) Secondary structures predicted using JPred

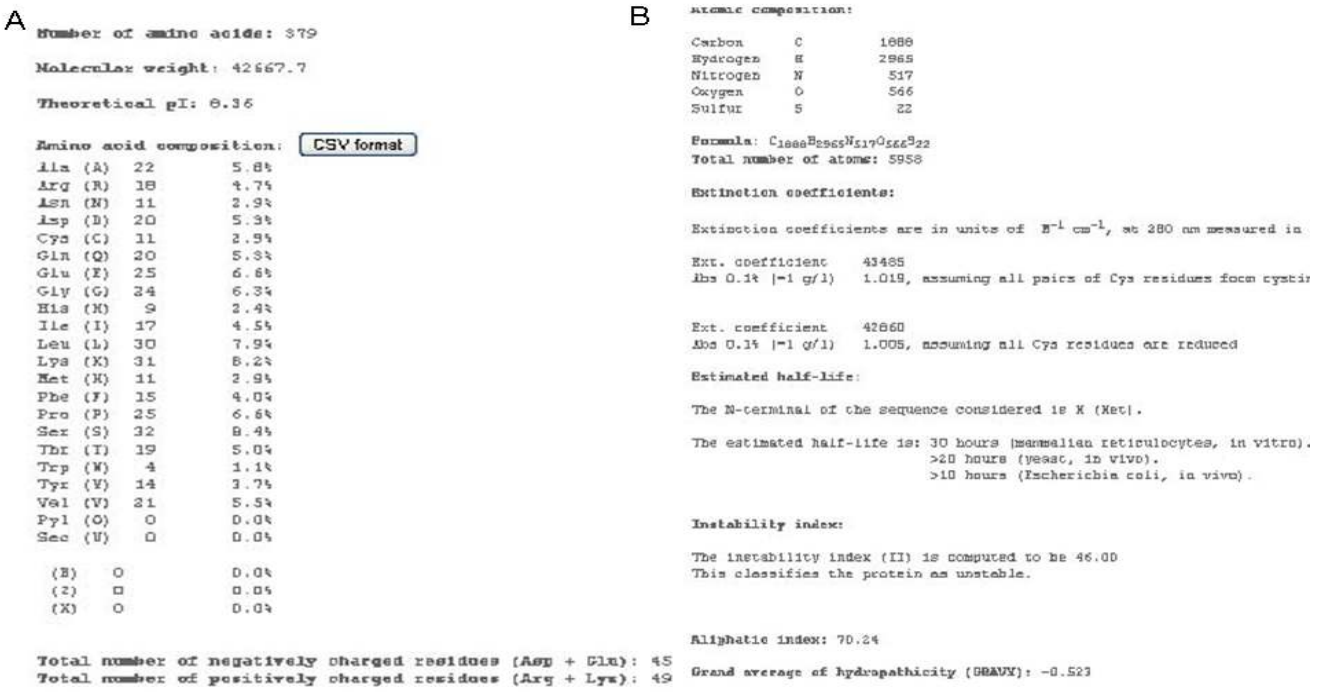


Fig 2: (**A**) Scalar parameters obtained from PROTPARAM, (**B**) Continued file of PROTPARAM showing other physico-chemical parameters.

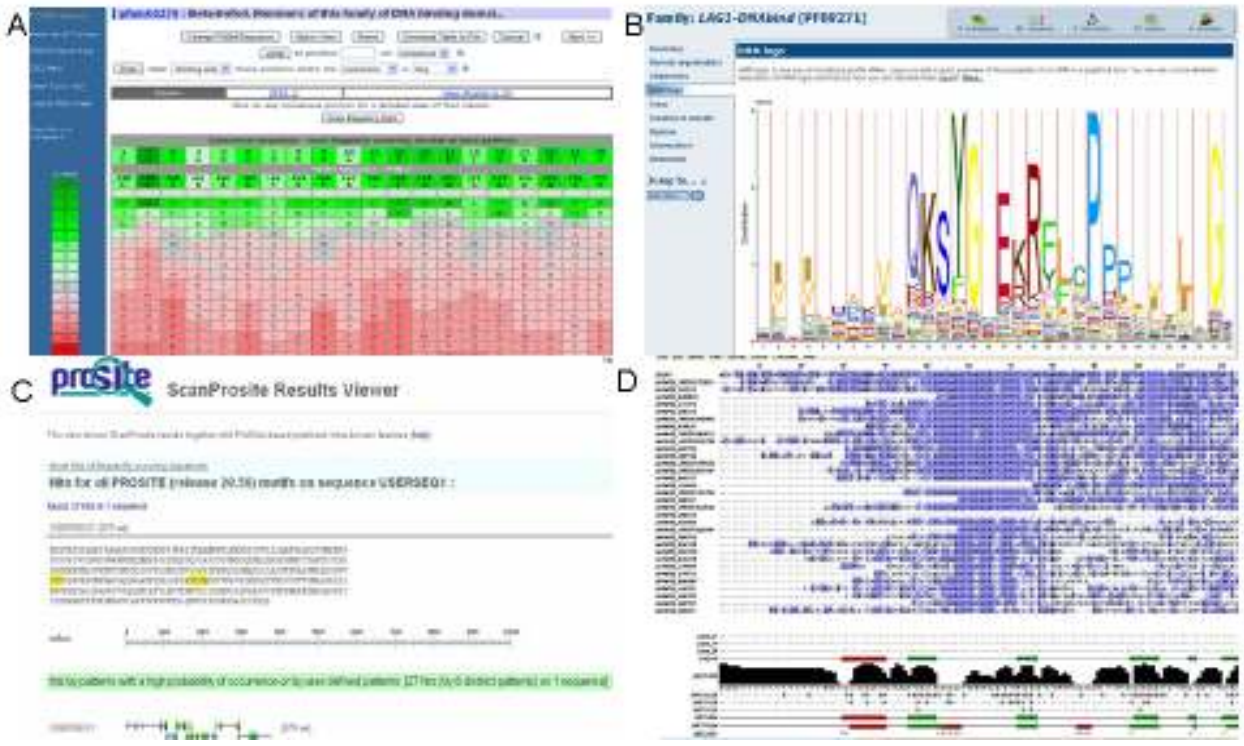
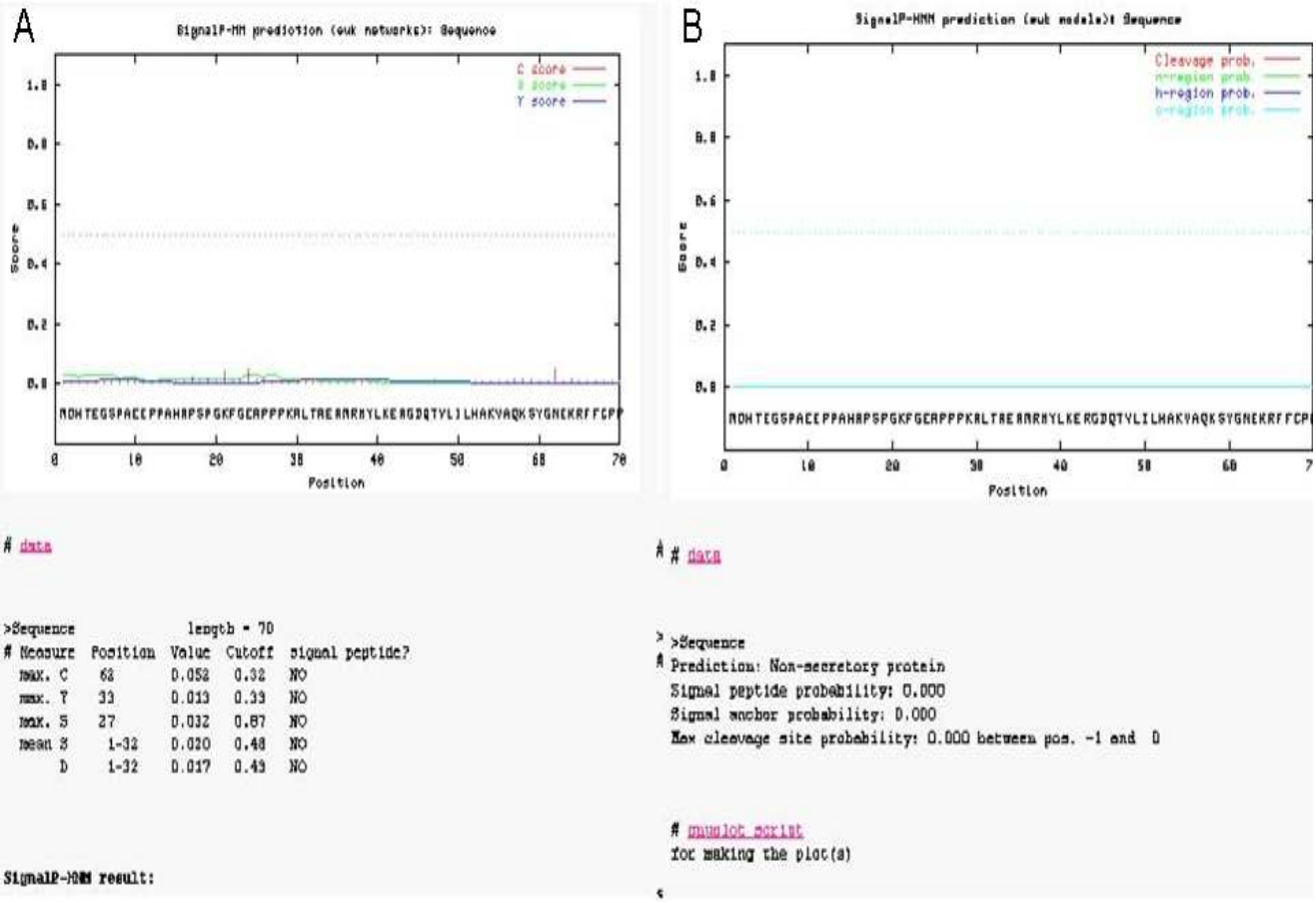


Fig 3: (A) Peptide cleavage sites predicted from SignalP using NN. (B) Peptide cleavage sites predicted from SignalP using HMM



AutoDock is molecular docking software which is one among common docking softwares used in major drug discovery grid projects. Autodock results for different compounds and RBP-J are shown in fig 4 and fig 5 which are considered to be the best conformer. We have run independent dockings using Autodock v 3.0 for each ligand. Results of Autodock calculations are collected, like binding energy, docking energy, inhibition constant, hydrogen bonds formed in that cluster is presented in table 2. The drawn chemical properties, PubChem ID and structures preferred for docking are tabulated in table 1. We were made sure that the compounds follow Lipinski's rule of 5 and docked with grid adjustments to get best conformation. From Fig 4 we can observe that Autodock best scored pose (fig 4D) for Ellagic acid, which has entered deep into the binding site. Fig 4A, 4B, 4C, 4E, 4F represents poses of compounds Scopoletin, Methyl brevifolin, Methyl gallate, Kaempferol and Quercetin respectively; we can see that in this case poses are not docked so deep as in previous case. Fig 5 represents pose of standard drug Naproxen, we can notice that in this case pose is not as deep as ellagic acid [11]. Results were compared for the Binding energy and number of hydrogen bonds formed and drawn in the form of graph (fig 6). Here Ellagic acid showed minimum binding energy (-7.65 g/mol) and as many as 7 hydrogen bonds, which are the properties of efficient drug. The compounds Methyl brevifolin and Quercetin showed better binding energy with nearly good number of hydrogen bonds. Where as Kaempferol showed moderate binding energy and only 3 hydrogen bonds were formed, which shows that amongst the 6 taken drugs it is less efficient. The standard drug Naproxen showed binging energy of -7.39 g/mol and formed 3 hydrogen bonds which is comparatively less than Ellagic acid. Hence, Ellagic acid is regarded as the candidate drug molecule for inhibition of Rheumatoid arthritis and steps can be taken forth to isolate this from the mentioned plant and develop a target based drug to RA. The final purpose is to reduce the number of targets for a good drug that has to be subjected to expensive and time-consuming synthesis and trialing.

Table 1: Chemical properties of the selected ligands along with PubChem ID and their structures

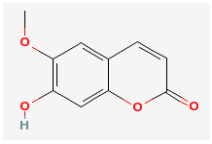
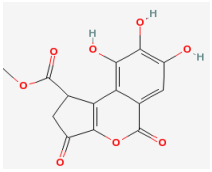
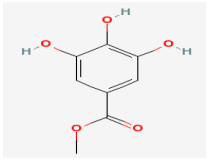
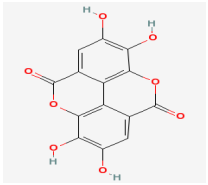
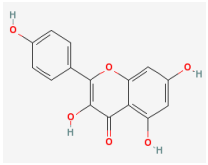
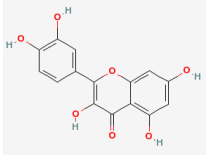
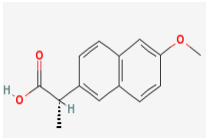
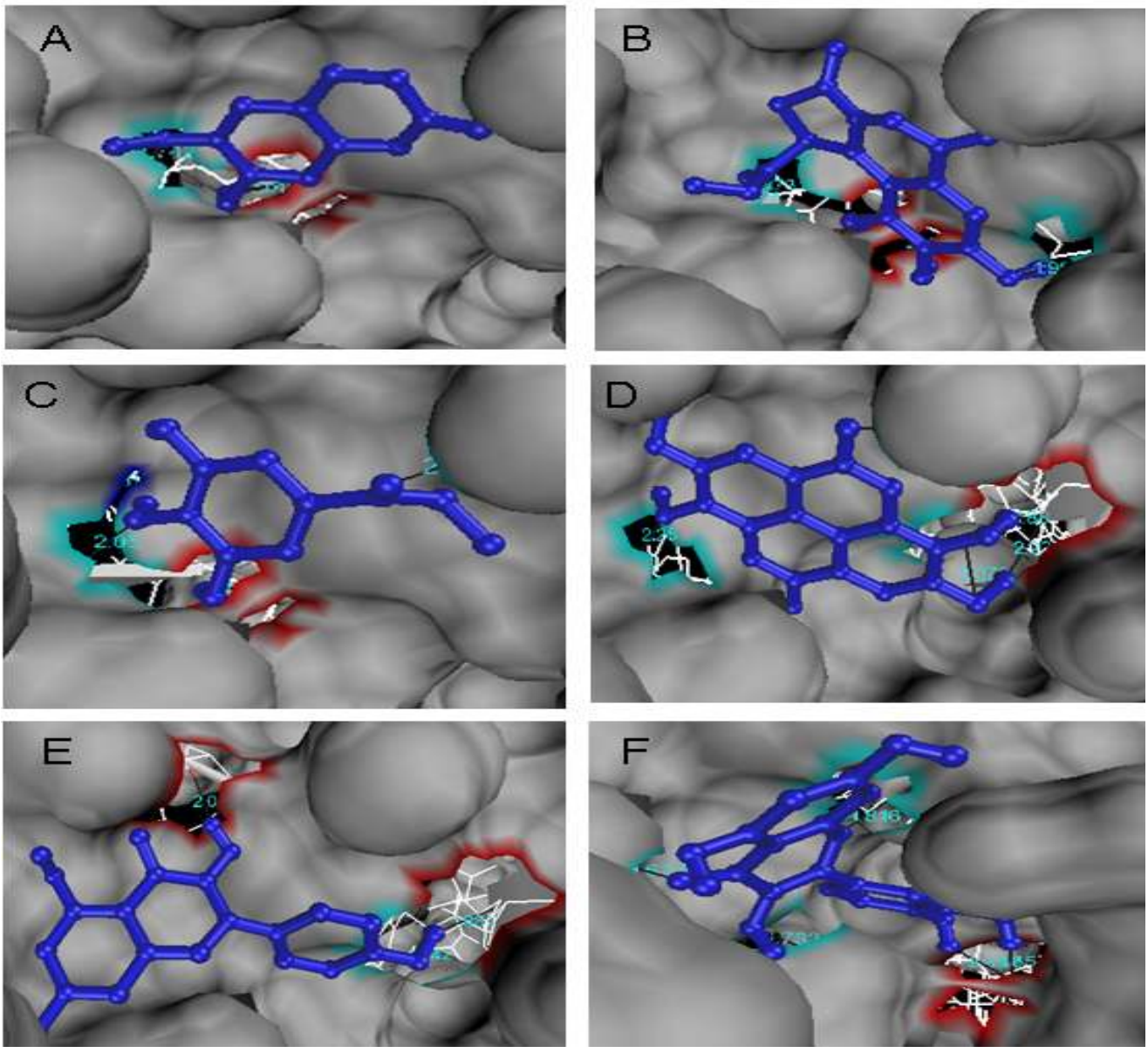
Sl. No	Name	CID	Mol.Wt. (g/mol)	Mol. Formula	LogP	H-Donor	H-Acceptor	Structure
1.	Scopoletin	5280460	192.16812	$C_{10}H_8O_4$	1.5	1	4	
2.	Methyl brevifolin	5319518	306.2244	$C_{14}H_{10}O_8$	-0.1	3	8	
3.	Methyl gallate	7428	184.14612	$C_8H_8O_5$	0.9	3	5	
4.	Ellagic acid	5281855	302.19264	$C_{14}H_6O_8$	1.1	4	8	
5.	Kaempferol	5280863	286.2363	$C_{15}H_{10}O_6$	1.9	4	6	
6.	Quercetin	5280343	302.2357	$C_{15}H_{10}O_7$	1.5	5	7	
7.	Naproxen	156391	230.25916	$C_{14}H_{14}O_3$	3.3	1	3	

Table 2: Docking results as obtained using Autodock V3.0.

Sl. No.	Ligand	Binding energy	Docking energy	Inhibition constant	No. of Hydrogen bonds formed
1	Scopoletin	-6.12	-6.34	3.25e-005	4
2	Methyl brevifolin	-7.16	-7.93	5.64e-006	6
3	Methyl gallate	-4.95	-5.72	0.000237	5
4	Ellagic acid	-7.65	-7.72	2.46e-006	7
5	Kaempferol	-6.38	-6.86	2.12e-005	3
6	Quercetin	-7.23	-7.78	5.06e-006	6
7	Naproxen	-7.39	-7.58	3.8e-006	3

Fig 4: Docking postures of (A) Scopoletin, (B) Methyl brevifolin, (C) Methyl gallate, (D) Ellagic acid, (E) Kaempferol and (F) Quercetin with RBP-J.



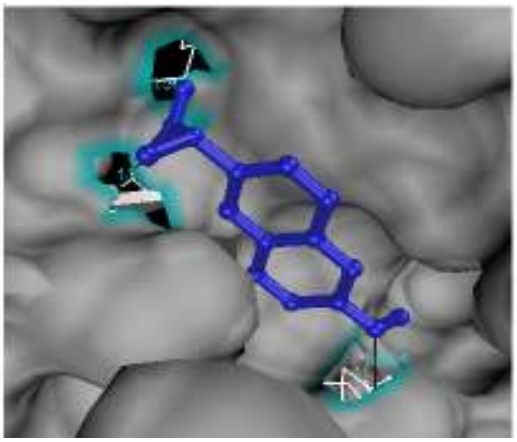
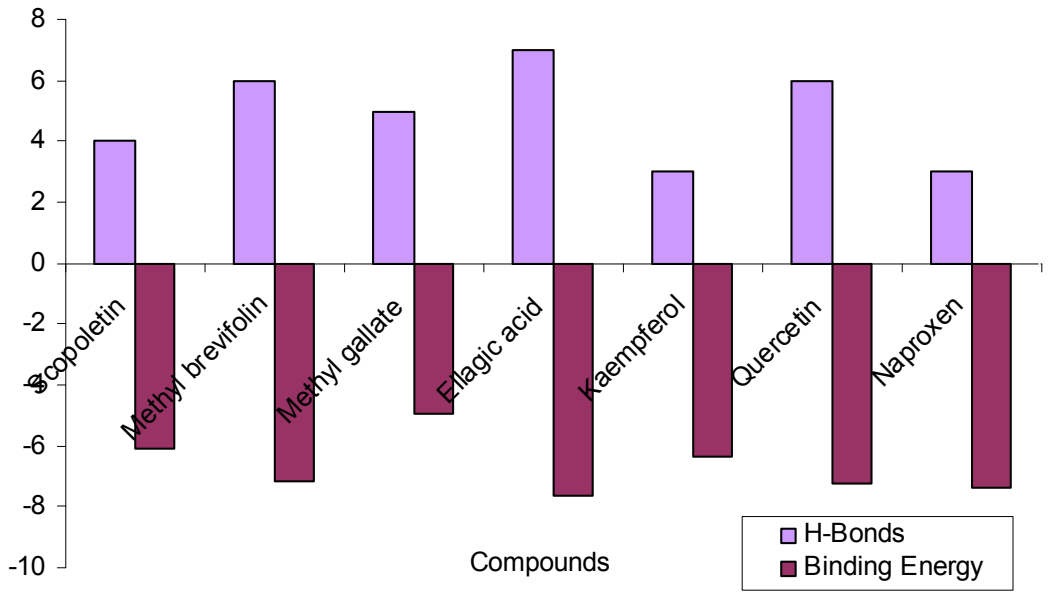


Fig 5: Docking posture of Naproxen with RBP-J.

Fig 6: Comparison of Hydrogen bonds formed and binding energy required.



CONCLUSION

Outcomes of these *in silico* investigations are an eye opener for the management of RA. *Invivo* anti-arthritic activities in pre-clinical and clinical level are now the realistic goal to achieve. Reaching early and sustained emission requires intensive therapy, monitoring of disease activity and structural damage to determine whether additional intervention is needed. Future efforts need to focus on the management of patients who are suffering and to achieve a permanent relief from side effects.

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