JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Aug. 2015. Vol. **9**, p. 07-15
SPECIAL ISSUE ON RECENT RESEARCH CHALLENGES IN BIO-MEDICAL APPLICATIONS

In Silico Approach Rendering Berberine as a Potent Anti-Inflammatory Drung

Tamilselvi Saravanakumar[1]*, N.M. Saravanakumar[2] and K.P. Kannan[3]

[1,3]Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam - 638452, TamilNadu, India.
[2]Department of Computer Science and Engineering, Bannari Amman Institute of Technology,Sathyamangalam - 638452, TamilNadu, India.

(Received: 11 March 2015; accepted: 02 May 2015)

Berberine has gained much attention in recent years owing to its multiple biochemical and pharmacological effects, including anticancer, antiviral, and antibacterial activities. The interaction profile of complex obtained on molecular docking using Glide module of Maestro Suite indicates berberine interacts at phenylvaline and valine residues of protein and has hydrophobic, non polar interaction and non-covalent interaction via hydrogen bonding at the mentioned active site residues of protein. The protein-ligand complex stability by performing molecular dynamics simulation run of the complex for 100 ps using MacroModel module of Schrodinger suite showed that the complex is stable with time evolution. The in silico study of protein-berberine complex rendered clear picture of interaction of berberine with the protein. Further detailed study of various derivatives of Berberine with protein might lead to more potent and novel class of PKC inhibitors.

Key words: p38 MAPK, anti-inflammatory, Molecular docking, Molecular dynamics (MD), Toxicity prediction, Berberine**.**

Medicinal plants have been explored therapeutically in traditional medicines and are a valuable source for drug discovery. There is a fact that botanicals mentioned in Ayurveda traditionally used for fever, wound-healing, edema and rheumatic diseases are sourced for anti-inflammatory properties (Tunon *et al*., 1995).

Herbal research using modern analytical and chemical techniques is needed to ensure efficacy and safety inorder to provide qualitative and quantitative analyses for dietary supplements, and to develop new, effective and safe excellent drugs. Insufficient knowledge about the molecular mechanism of the components of medicinal plants limits the scope of their application and hinders the effort to design new drugs using the therapeutic principles of herbal medicines. This problem can be partially elevated with the introduction of efficient methods for rapid identification of protein targets of herbal ingredients. Presently efforts are directed at developing efficient computer methods for facilitating target identification (Chen *et al*., 2003). The computing resources considered included the various databases currently available and the software that has been or might be used to analyse these data. The various different kinds of databases identiûed as relevant included those holding ethnobotanical, chemical, pharmacological and/or toxicological data on the herbs used in medicine, as well as those that reveal potential molecular targets for the herbal constituents. The software tools considered as relevant included programs that provide for (a) Virtual screening of natural product libraries and chemical libraries, (b) pattern recognition and (c) bioinformatics tools.

Drug design is an iterative process revealing lead structures, which can be chemically

* To whom all correspondence should be addressed.
Tel.: +91 4295 226000; Fax: +91 4295 226666;
E-mail: tmlsaran@gmail.com

modified and improved through knowledge of structure-activity relationship, mechanism of action, drug metabolism, molecular modelling and combinatorial chemistry studies. Finally, efficacy and toxicity determination as well as clinical trials can contribute to the generation of new drugs from medicinal plants. To continue the legacy of herbal medicines, as well as the worldwide uses of other medicinal herbs, continued investigation of active formulations, bioactive fractions, and isolated compounds is critical in the 21st century (Lee, 2003). Several studies dealing with the virtual screening (VS) of constituents have employed various computational methods to identify potential ligands for protein targets of pharmacological/therapeutic interest (Huang *et al*., 2007). The screening techniques employed include pharmacophore search, molecular docking, and screening. Even though the introduction of omics techniques in herbal research is relatively recent and not yet widely disseminated among scientists in the ûeld, meaningful results are suggestive of a great potent application of omics and related bioinformatic techniques in the study of syndromes and phytocomplexes from traditional Ayurvedic medicine.

Inflammation is one of the characteristic features of immune mediated protection against allergens or tissue mediated responses. Chronic inflammation arises due to aberrant or unchecked immune responses. Inflammation has also been a contributing factor in various disorders (Nathan, 2002) and diseases including cancer (James *et al*., 2005; Coussens and Werb, 2002). Recent advances in anti-inflammatory research include the study to identify potential anti-inflammatory molecular targets and regulation of inflammation signalling pathways. Mitogen activated protein kinases (MAPK) , mainly used as an anti-inflammatory target as they are signaling molecules in cytokine synthesis, are of several classes such as c-jun N-terminal kinase, p38 MAP kinase and extracellular signal–regulated kinase. The pathophysiological and biological roles of p38 signalling molecules are reviewed to have an increasing complex signaling network leading to their activation (Schindler *et al*., 2007).

As an alkaloid, Berberine has a definite potent ability of a drug as it posseses diverse pharmacological properties such as anti hypercholesterimic (Weijia *et al*., 2004), dietary therapy conjestive heart failure (Dhar, 1980), antioxidant, activator of protein kinases, antirrhythmic activity, antiproliferative and antimigratory properties (Singh *et al*., 2010). Use of MAPK inhibitors emerges as an attractive strategy as they are capable of reducing both the synthesis of pro-inflammatory cytokines and their signalling. The p38 MAP kinases are a family of serine/threonine protein kinases playing important roles as stress signals. Inhibition of these stress signals has shown anti-inflammatory responses by inhibiting the inflammatory mediators. The present study evaluates the effectiveness of berberine on MAPK and its identity to be a novel drug to provide a framework of its activity in the inflammatory pathway by exploring the anti-inflammatory potency of berberine by targeting Mitogen-Activated Protein Kinases computationally.

MATERIALS AND METHODS

Protein and ligand selection and source

In this study, p38 Mitogen-Activated Protein Kinase (MAPK) metabolic pathway was observed from KEGG Reference pathway. p38 MAPKs are members of the MAPK family that are activated by a variety of environmental stresses and inflammatory cytokines. Stress signals are delivered to this cascade by members of small GTPases of the Rho family (Rac, Rho, Cdc42). As with other MAPK cascades, the membrane-proximal component is MAPKKK, typically a MEKK or mixed lineage kinase (MLK). The MAPKKK phosphorylates and activate MKK3/5, the p38 MAPK kinase. MKK3/6 can also be activated directly by ASK1, which is stimulated by apoptotic stimuli. p38 MAPK is involved in regulation of Hsp27 and MAPKAP-2 and several transcription factors including ATF2, STAT1, THE Max/Myc complex, MEF-2, ELK-1 and indirectly CREB via activation of MSK1. The interrelated enzyme kinetics linked to p38 protein domain annotation was retrieved. The 3-D crystal structure of p38 MAPK protein complex with 5-(2-chlorophenyl)-n-(5-(cyclopropylcarbamoyl)-2-methylphenyl)-2-thiophenecarboxamide (co-crystal ligand) bearing PDB ID: 4KIN (Wrobleski *et al*., 2013) as obtained from open source Protein

Data Bank database (Sussman *et al*., 1998) (Fig 1). The crystal structure of Berberine compound (CID: 2353) and Diclofenac (CID: 3033) was obtained from open source NCBI-PubChem compound database (Fig. 1) (Wang *et al*., 2009; Sayers *et al*., 2009).

Protein stability

The structural variation analysis of the retrieved p38 MAPK protein is of prime importance in order to check its stability under given condition. The stability of retrieved protein was studied by performing molecular dynamics simulation using MacroModel module of Schrodinger suite. Minimization parameters of 5000 maximum iterations and 0.05-convergence threshold were set. A total trajectory of 1000 samples in water solvent at 300 K for total 1 ns simulation run was generated. The root mean squared deviation (RMSD) which is a measure of the scalar distance between atoms of the conformational structures of protein, of each trajectory were super positioned and displacement of each conformation from its native position was analysed.

Molecular docking

A thorough understanding of protein and compound interaction is of prime importance in order to further acquire an exact compound pose reacting at the protein-binding pocket that is a key to achieve a ligand governing protein function. This is attained by docking all possible conformation of selected ligands with protein/ receptor. In present study, the co-crystal ligand, Berberine and Diclofenac, an anti-inflammatory and analgesic compound readily available in market were docked with p38 MAPK protein using Glide module of Schrodinger suite (Friesner *et al*., 2004). Among all available conformations and orientations of ligand, a pose that fits well to the active binding site was examined. Hence, prior to docking, all possible stereoisomers, tautomers, ionisation states of ligands were generated by Epik module (Greenwood *et al*., 2010; Shelley *et al*., 2007) using Ligprep tool of Schrodinger suite. The retrieved crystal structure of protein was preprocessed using PrepWizard tool of Schrodinger suite that adds hydrogen, assigns bond-order, deletes heteroatoms and performs protein optimization. The crystal structure of p38 MAPK protein obtained from PDB database is a complex with 5-(2- chlorophenyl)-n- (5-(cyclopropylcarbamoyl)-2- methylphenyl)-2-thiophenecarboxamide having four chains (A, B, C and D), four hetero atoms, missing side chains and 16 water molecules. The protein crystal structure was refined by removing chains B, C and D and adding missing side chains, removing heteroatoms and water molecules. A grid for receptor centred at all residues was generated of 20, 20 and 20 Å (x, y and z) dimension using which an extra precision, flexible ligand docking was performed.

Post docking evaluation

An atomistic examining of protein-ligand site-site interaction provides details of the interacting residues of protein and atoms of ligands. A ligand conformation and orientation highly interacting at the active binding site of the protein was determined based on the GlideScore. GlideScore is obtained by solving a multi ligand scoring function, which combined with the energy model ranks the ligand poses by summing GlideScore, non-bonded interaction energy, excess internal energy; in case of flexible ligand docking and hydrogen bond energy.

Molecular dynamics of protein-ligand complex and its stability

Molecular dynamics simulation of a system provides understanding of the behaviour of the system with time. Hence, a molecular dynamics simulation of best ligand pose and protein complex obtained after molecular docking was performed for 100 ps using Macromodel module of Schrodinger suite (Macromodel, 2013). The structure was minimized to a low gradient using PRCG (Polak-Ribiere Conjugate Gradient) method with 1000 maximum iterations and at 0.05 convergence threshold. The Potential energy calculation was performed using OPLS_2005 force field (Jorgensen *et al*., 1988) by applying no constraints to atoms, bonds, angle and dihedral. The charges were taken from OPLS_2005, Van der Waals and electrostatic calculation were performed at extended cutoff. Total trajectories were generated for 100 samples. An equilibration run for 1 ps and total simulation run for 100 ps was performed at 300 K temperature in water solvent.

Toxicity Prediction

The Biophysical and Pharmaceutical properties (Absorption, Distribution, Metabolism and Excretion) of Berberine was determined and analysed using Qikprop tool of Schrodinger suite.

Qikprop tool accurately predicts molecular properties of compound and provides valid range for each property by comparing with 95% known drugs.

RESULTS

Protein stability analysis

Molecular dynamics study provided information regarding conformational variation of the protein with respect to its reference structure (Fig 3). Sampling of 1000 conformational variant structure generated during molecular dynamics simulation of p38 MAPK protein showed that the protein is stable under physiological pH value of ± 7.0 and at 300 K temperature with root mean squared deviation of 1.799 Å (Fig. 2).

Molecular docking analysis

Molecular interaction study of the co-crystal ligand, Berberine and Diclofenac revealed the binding modes as well as binding ability of compounds with the p38 MAPK protein. The pictorial representation of interacting sites of protein-ligand complex is shown in Fig. 4, 5 and 6 whereas the two dimensional spatial interaction of ligands with protein residues is shown in Fig. 7, 8 and 9.

Ligand based molecular docking of ligands with protein showed that the Diclofenac compound showed only one hydrogen bond (1.64 Å) interaction through ASP residue of protein with the total GlideScore of -3.65 kcal/mol (Fig. 6). The co-crystal ligand interacted with the protein by forming two weak hydrogen bonds(2.04 Å and 2.38

Table 1. The predicted molecular properties values for the co-crystal ligand, Diclofenac and Berberine

Property	Co-crystal ligand	Diclofenac	Berberine	Recommended Range
CNS activity	0	-1	1	-2 to +2
Molecular weight	427.044	296.15	337.37	130.0-725.0
SASA	768.31	502.61	552.48	300.0-1000.0
FOSA	630.36	34.086	326.61	0.0-750.0
FISA	68.87	90.76	1.546	7.0-330.0
PISA	0	271.968	224.322	0.0-450.0
Volume	1389.834	863.074	995.312	500.0-2000.0
donorHB	2	2	0	0.0-6.0
accptHB	5.5	2.5	4	2.0-20.0
dip^2/V†	0.0426	0.0128	0.0171	0.0-0.13
ACxDN^.5/SA	0.010	0.007	0	0.0-0.05
QPlogPC16	12.669	10.023	9.132	4.0-18.0
QPlogPoct	21.604	14.019	13.183	8.0-35.0
QPlogPw	13.079	7.942	6.084	4.0-45.0
QPlogPo/w	4.069	4.497	4.092	-2.0-6.5
QPlogS	-6.17	-5.36	-4.27	-6.5-0.5
QPlogHERG	-2.28	-3.089	-4.663	Concern below -5
QPlogCaco-2	913.09	345.80	9577.26	<25 poor, >500 great
QPlogBB	-0.26	-0.21	0.43	-3.0-1.2
QPPMDCK	2775.18	758.30	5687.95	<25 poor, >500 great
QPlogKp	-2.41	-1.85	-0.57	-8.0- -1.0
QPlogKhsa	0.438	0.059	0.352	-1.5-1.5
Human OralAbsorption	3	3	3	1 is low, 2 is medium and 3 is high
Percent Human- Oral Absorption	100	100	100	<25% poor, >80% high
PSA	67.43	57.89	33.51	7.0-200.0
Lipinski's RO5 violation	0	0	0	Maximum is 4
Jorgensen's RO3 violation	1	0	0	Maximum is 3
Jm (Predicted maximum transdermal transport rate)	0.001	0.301	4.962	-

Å) with GLU and THR residues and one strong hydrogen bond (1.96 Å) with ASP residue of the protein (Fig. 4). Berberine compound showed two hydrogen bond (1.998 Å and 1.995 Å) interactions with hydrogen bond donating ARG residue of protein via hydrogen bond accepting methoxy group of Berberine (Fig. 6). Berberine showed strong binding due to the presence of two-methoxy group that formed hydrogen bond with the ARG residue of the protein. The conformation and orientation of Berberine after fitting at the interaction site of protein gave total Glidescore of

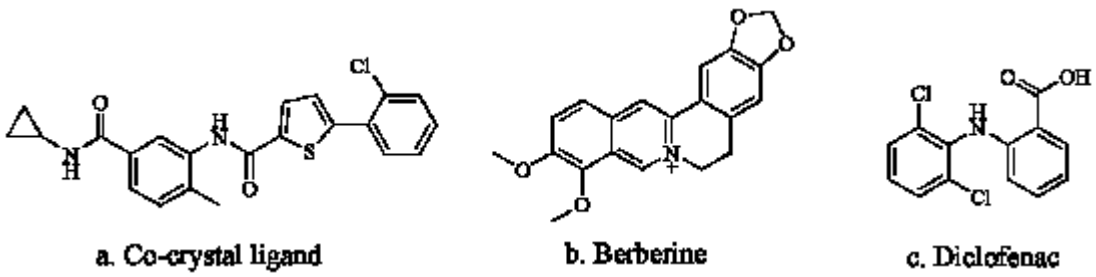

Fig. 1. Chemical structure of ligands

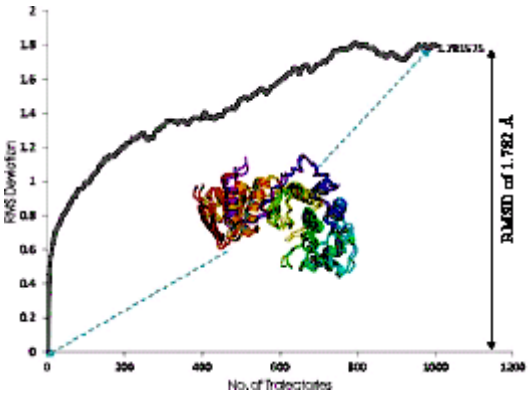

Fig. 2. RMSD plot of p38 MAPK protein superpositioned structure after 1 ns molecular dynamics simulation run

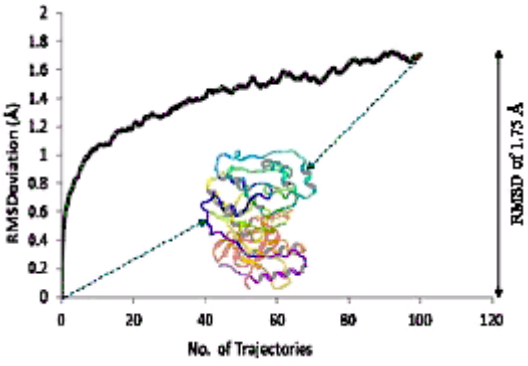

Fig. 3. RMSD plot for super positioned structures of p38 MAPK protein- Berberine complex after 1 ns molecular dynamics simulation run

Fig. 4. Interaction profile of p38 MAPK protein-co-crystal ligand complex

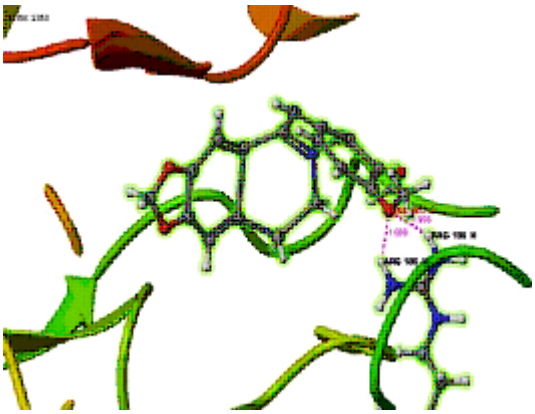

Fig. 5. Interaction profile of p38 MAPK protein–Berberine complex

-9.0 kcal/mol that is higher than the co-crystal ligand and Diclofenac.

Protein-ligand complex stability

Molecular dynamics simulation of the protein and Berberine complex for 100 ps showed that the complex attains stability at 60 ps and remains stable throughout the simulation run. It can be observed from the root mean squared deviation (RMSD) plot (Fig. 3) that the conformational variant structures fluctuates at RMSD of 1.75 Å from its native structural state. The stability of complex under given physiological conditions (pH ± 7 and 300 K) confirmed that there is no structural variation or deformation in the protein after Berberine binds to it and the binding mode as well as binding ability of Berberine remains unaffected with minute protein conformational variation.

Toxicity prediction analysis

Through molecular docking and molecular dynamics simulation, Berberine was found to be showing best binding affinity and stability at pH ± 7 and 300 K. The molecular properties of the co-crystal ligand, Berberine and Diclofenac are included in Table 1. Observations indicated increasing drug like properties such as Solvent Accessible Surface Area (SASA), Partition coefficient, aqueous solubility, Central Nervous System (CNS) activity, Blood-brain barrier coefficient, Human oral absorption, Lipinski's (rule of five) RO5 violation, no Jorgensen rule of three (RO3) violation and predicted maximum transdermal transport rate for Berberine as compared to Diclofenac, although the hydrophilic solvent accessible area (FISA) of Berberine was too low and out of recommended range. This is due to the

Fig. 6. Interaction profile of p38 MAPK protein-Diclofenac complex

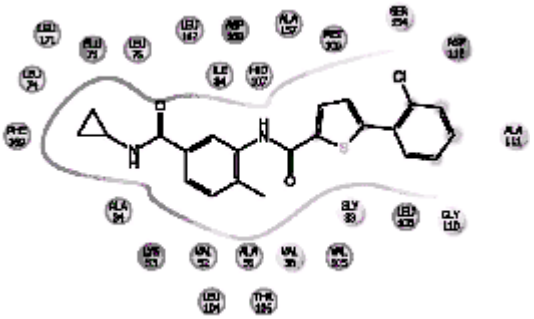

Fig. 7. Spatial interaction of co-crystal ligand with residue of p38 MAPK protein

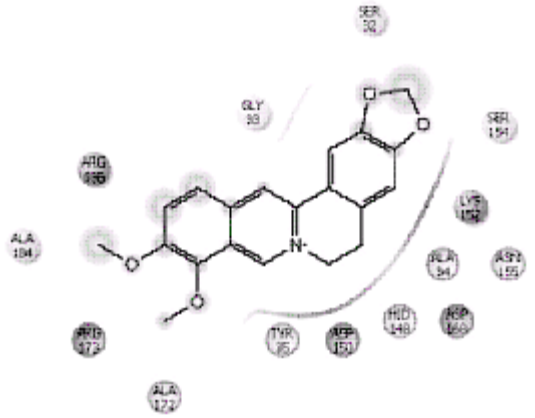

Fig. 8. Spatial interaction of Berberine with residue of p38 MAPK protein

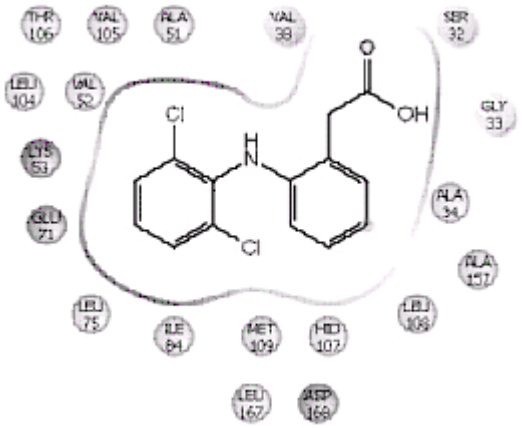

Fig. 9. Spatial interaction of Diclofenac with residue of p38 MAPK protein

absence of exposed heteroatom other than oxygen atoms of dioxobenzene ring. The Predicted octanol/ water partition coefficient is negligibly lower than Diclofenac but in expected range. The co-crystal possess better drug like properties overall but the predicted Caco-2 cell permeability, predicted skin permeability, predicted Madin-Darby canine kidney (MDCK) permeability and Predicted maximum transdermal transport rate of Berberine was found to be higher than the co-crystal ligand as well as Diclofenac.

DISCUSSION

Berberine, a well known anti-inflammatory compound was studied extensively in vitro and invivo for its efficacy and safety. Anti-inflammatory mechanism of berberine still remains an unclear picture and is yet to be resolved. In silico studies are versatile in identifying the possible targets in the inflammatory cascade for Berberine, to bind and exert its antiinflammatory activity. To our knowledge, this is the first study aimed at docking studies for berberine with MAPK as anti-inflammatory targets. The docking studies provided good insights into the binding of berberine at the molecular level, to different MAP kinase, which are proven to play a major role in inflammatory cascade.

There are literature cited regarding the inhibition of the transcription factors, cytokines and other enzyme involved in inflammatory pathway. The anti-inflammatory activity of berberine was also associated with its inhibitory effect on the mitogen-activated protein kinase (MAPK) signaling pathways, which were activated by inflammatory stimuli. The inhibitory effect of berberine on MAPKs was dependent on AMPK activation in macrophages (Jeong *et al*., 2009). It seems that conflicting results exist concerning the regulatory effect of berberine on MAPK signaling. Although some results suggested that berberine suppressed inflammation through inhibiting MAPKs, others indicated that P38 was activated by berberine which was considered important for efficacy of berberine against oxidative stress and inflammation (Wang *et al*., 2012; Mima, 2013). In reviewing published results, it seemed that berberine could increase P38 phosphorylation in unstimulated cells with baseline P38 activity but decrease MAPK phosphorylation in cells treated with inflammatory stimuli like lipopolysaccharide (LPS), FFA and TNF-á which could activate MAPKs (Wang, 2014). In the current study, the targeted anti-inflammatory potency of berberine on p38 MAPK is evident by the docking study results revealing that berberine can be a potent inhibitor of MAPK signalling mechanism although the mechanism is to be further confirmed.

A strong relationship between p38 MAP Kinase pathway, ERK and PKC with inflammation has been well established and are postulated to regulate inflammatory condition in rheumatoid arthritis, Alzheimer's disease and inflammatory bowel disease. The activation of these signaling kinases plays essential roles in the production of pro-inflammatory cytokines (IL-1â, TNF- á and IL-6), induction of enzymes such as COX-2, iNOS and induction of VCAM-1 and other adherent proteins along with other inflammatory molecules (Zarubin and Han, 2005). Diclofenac is a nonsteroidal anti-inflammatory drug, which is available as prescription (RX) and over-the-counter (OTC) medication for the systemic and topical treatment of painful and inflammatory conditions. Invivo and pharmokinetic studies indicate confirmation of the anti-inflammatory and analgesic effects of this drug preferentially distributed in inflamed tissues (Li *et al*., 2007; Elhenawy *et al*., 2014). The stability and toxicity analysis of berberine against diclofenac showed berberine to have stabilized in the binding pocket of MAPK and the better therapeutic index indicates higher safety for its use.

Although some of the key issues of BBR in reducing oxidative stress and inflammation still needs to be further studied especially targeting regulation of UCP2, MAPKs, and PPARã by berberine, it is quite clear that on comparison with diclofenac, berberine may be targeted for its extensive use as a anti-inflammatory drug from herbal sources. Further investigation of the influence of berberine on anti-inflammatory signalling pathways will help to clarify the pharmacology of berberine against inflammation and promote the research and development of anti-inflammatory natural products.

CONCLUSION

An in-silico approach undertaken in order to investigate the binding, molecular toxicity property of the selected compounds. The molecular docking study predicted Berberine binds well at the active binding site as compared to the co-crystal ligand and Diclofenac with the GlideScore of -9.0 kcal/mol, -5.94 kcal/mol and -3.65 kcal/mol respectively, which is due to the presence of two hydrogen bond accepting methoxy functional group that exhibits strong hydrogen bond interaction with ARG protein residue. The molecular dynamics simulation of the protein-Berberine complex confirmed that the protein is stable with RMSD of 1.75 Å. The Biophysical and Pharmaceutical molecular properties determined through toxicity prediction showed that Berberine possessed better drug like properties than Diclofenac, which may be a future market competitor for Diclofenac as an anti-inflammatory drug.

REFERENCES

1. Chen, X., Ung, C.Y., Chen, Y., Can an in silico drug-target search method be used to probe potential mechanisms of medicinal plant ingredients? *Nat Prod Rep*, 2003; **4**: 432-44.
2. Coussens, L.M., Werb, Z., Inflammation and cancer. *Nature*, 2002; **420**(6917), 860-7.
3. Dhar, J., Berberine and oriental sore. *Ind. J. Dermatol. Vernereol. LeP.*, 1980; **46**: 163.
4. Elhenawy, A.A., El-Gazzar, M.A., Mohmmed, H.M., Synthesis, anti-Inûammatory, Analgesic, Molecular Modeling and ADMET Studies of Novel Diclofenac Derivatives Containing Alanyl Moiety. *Chemistry and Materials Research*, 2014; **6**(2), 69-77.
5. Friesner, R.A., Banks, J.L., Murphy, R.B., Halgren, T.A., Klicic, J.J., Mainz, D.T., Repasky, M.P., Knoll, E.H., Shaw, D.E., Shelley, M., Perry, J.K., Francis, P., Shenkin, P.S., Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. *J. Med. Chem*, 2004; **47***:* 1739–1749.
6. Greenwood, J.R., Calkins, D., Sullivan, A.P., Shelley, J.C., Towards the comprehensive, rapid, and accurate prediction of the favorable tautomeric states of drug-like molecules in aqueous solution. *J. Comput. Aided Mol. Des*, 2010; **24**: 591-604.
7. Huang, Q., Qiao, X.B., Xu, X.J., Potential synergism and inhibitors to multiple target enzymes of Xuefu Zhuyu decocotion in cardiac disease therapeutics: a computational approach. *Bioorganic & Medicinal Chemistry Letters*, 2007; **17**: 1779–1783.
8. James, V., Corino, G., Robertson, T., Dutton, N., Halas, D., Boyd, A., Bentel, J., Papadimitriou, J., Early diagnosis of breast cancer by hair diffraction. *Int. J. Cancer*, 2005; **114**: 969–972.
9. Jeong, H.W., Hsu, K.C., Lee, J.W., Berberine suppresses proinflammatory responses through AMPK activation in macrophages. American *Journal of Physiology - Endocrinology and Metabolism*, 2009; **296**(4), E955–E964.
10. Jorgensen, W.L., Tirado-Rives, J., The OPLS [optimized potentials for liquid simulations] potential functions for proteins, energy minimizations for crystals of cyclic peptides and crambin, *J Am Chem Soc*, 1988; **110***:* 1657-1666.
11. Lee. K.H., Research and future trends in the pharmaceutical development of medicinal herbs from Chinese medicine. *Public Health Nutr*, 2003; **4**(A), 515-22.
12. Li, L., Rossoni, G., Sparatore, A., Lee, L.C., Del Soldato, P., Moore, P.K., Anti-inflammatory and gastrointestinal effects of a novel diclofenac derivative. *Free Radic Biol Med*, 2007; **42**(5): 706-19.
13. Mima, A., Inflammation and oxidative stress in diabetic nephropathy: new insights on its inhibition as new therapeutic target. *Journal of Diabetes Research.* 2013; (248563), 8.
14. Nathan, C., Points of control in inflammation. *Nature*, 2002; **420**(6917), 846-52.
15. Sayers, E.W., Barrett, T., Benson, D.A., Bolton, E., Bryant, S.H., Canese, K., Chetvernin, V., Church, D.M., DiCuccio, M., Federhen, S,, Feolo, M., Fingerman, I.M., Geer, L.Y., Helmberg, W., Kapustin, Y., Landsman, D., Lipman, D.J., Lu, Z., Madden, T.L., Madej, T., Maglott, D.R., Marchler-Bauer, A., Miller, V., Mizrachi, I., Ostell, J., Panchenko, A., Phan, L., Pruitt, K.D., Schuler, G.D., Sequeira, E., Sherry, S.T., Martin, S.M., Sirotkin, K., Slotta, D., Souvorov, A., Starchenko, G., Tatusova, T.A.,Wagner, L., Wang, Y., Wilbur, W.J., Yaschenko, E., Ye, J., 2009. Database Resources of the National Center for Biotechnology Information. Nucleic Acids Res. 37, D216 - D223
16. Schindler, J.F., Monahan, J.B., Smith, W.G., p38 pathway kinases as anti-inflammatory drug targets. *J Dent Res*, 2007; **86**: 800–811.
17. Shelley, J.C., Cholleti, A., Frye, L., Greenwood,

J.R., Timlin, M.R., Uchimaya, M., Epik : a software program for pK_a prediction and protonation state generation for drug-like molecules. *J. Comp.-Aided Mol. Design*, 2007; **21***:* 681-691.

18. Singh, A., Sanjiv, D., Kaur, N., Jaswinder, S., Berberine: Alkaloid with wide spectrum of pharmacological activities. *Journal of Natural Products*, 2010; **3**: 64-75.
19. Sussman, J.L., Lin, D., Jiang, J., Manning, N.O., Prilusky, J., Ritter, O., Abola, E.E., Protein Data Bank (PDB): database of three-dimensional structural information of biological macromolecules. *Acta Crystallogr. D Biol. Crystallogr*, 1998; **54**: 1078–1084
20. Tunón, H., Olavsdotter, C., Bohlin, L., Evaluation of anti-inflammatory activity of some Swedish medicinal plants: Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. *J Ethnopharmacol*, 1995; **48**: 61–76.
21. Wang, Q., Qi, J., Hu, R., Chen, Y., Kijlstra, A., Yang, P., Effect of berberine on proinflammatory cytokine production by ARPE-19 cells following stimulation with tumor necrosis factor-á. Investigative Ophthalmology & Visual Science, 2012; **53**(4): 2395–2402.
22. Wang, Y., Xiao, J., Suzek, T.O., Zhang, J., Wang, J., Bryant, S.H., PubChem: a public information system for analyzing bioactivities of small molecules. *Nucleic Acids Res*, 2009; **37**: W623–W633.
23. Wang, Y., Attenuation of berberine on lipopolysaccharide-induced inflammatory and apoptosis responses in â-cells via TLR4-independent JNK/NF-êB pathway. *Pharmaceutical Biology*, 2014; **52**(4), 532-538
24. Weijia, K., Jing, W., Parveen, A., Meihong, L., Satoru, I., Cong, L., Yanling, W., Zizheng, W., Shuyi, S., Huaining, P., Shukui, W., Jingdan, W., Yue, W., Zhuorong, L., Jingwen, L., Jian-Dong, J., Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat. Med*, 2004; **10**: 1344–1351.
25. Wrobleski, S.T., Lin, S., Dhar, T.G., Dyckman, A.J., Li, T., Pitt, S., Zhang, R., Fan, Y., Doweyko, A.M., Tokarski, J.S., Kish, K.F., Kiefer, S.E., Sack, J.S., Newitt, J.A., Witmer, M.R., McKinnon, M., Barrish, J.C., Dodd, J.H., Schieven, G.L., Leftheris, K., The identification of novel p38á isoform selective kinase inhibitors having an unprecedented p38á binding mode, *Bioorg Med Chem Lett*, 2013; **23**(14); 4120-6
26. Zarubin, T., Han, J., Activation and signaling of the p38 MAP kinase pathway. *Cell Research*, 2005; **15**: 11–18.