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Original Article

TARGETING *JATROPHA* DERIVED PHYTOCHEMICALS TO INHIBIT THE XANTHINE OXIDASE & CYCLOOXYGENASE-2: *IN SILICO* ANALYSIS TOWARDS GOUT TREATMENT

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ABSTRACT

Objective: Gouty arthritis is a well known disease with an abrupt attack causing extreme pain in and around the joints. Accumulated urate crystals, being the reason of the disease cause a lot of inflammation leading to swelling in the joints. These diseases are being treated using NSAIDs, Colchicine as well as few of the Glucocorticoids but these have some unnatural effects primarily, gastrointestinal and cardiovascular side effects. Nowadays, *Jatropha curcas*, a medicinal plant has been studied for anti – inflammatory properties therefore the phytochemical constituents of this plant can be an effective drug against this Gout disease.

Methods: Herein for docking, Lamarckian Genetic algorithm was applied using Autodock4.2 (version 1.5.6). Xanthine Oxidase and Cyclooxygenase-2 proteins from *Homo sapiens* were modeled using the Swiss model and screened against the phytochemicals from *Jatropha* species.

Results: The results demonstrated that Jatrophone (KNApSAcK_ID: C00003446), 6β-hydroxy-4-stigmasten-3-one (KNApSAcK_ID: C00029573) and Palmarumycin CP1 (KNApSAcK_ID: C00035859) had a good affinity for both, Xanthine oxidase and COX-2. Further, the interaction profile of the phytochemicals with both the protein was analyzed using LigPlot+.

Conclusion: The interaction pattern phytochemicals with the Xanthine Oxidase and Cyclooxygensae-2 may provide hints for the design of novel derivatives with higher potency and specificity.

Keywords: Gout, Xanthine Oxidase, Cyclooxygenase-2, *Jatropha*, Phytochemicals, Autodock 4.2.

INTRODUCTION

Prevalence of an inflammatory arthritis, widely known as Gout, has been increased lately and was found to be the highest in countries like USA, UK and China [1-2]. This disease has been known to cause great impact on people but most of the cases remain untreated. Gout is basically a joint disease, with the most common and sudden symptoms that includes lot of pain, swelling and redness. The main reason behind this disease is the accumulation of uric acid in blood. Uric acid, being a waste product is produced in the body after the breakdown of purines and since it is a waste, it is excreted out through the kidneys in a normal healthy human being. Thus, the body maintains its balance.

When the balance is disturbed, that is, uric acid produced in the body is either not excreted or excreted very little; it accumulates in form of sodium salts in and around joints. These restrict the movements and cause severe pain. The urate crystals (Monosodium urate or MSU) are hygroscopic and bind different proteins including the immunoglobulin G and complement proteins, thereafter interacting with some specific receptors on leukocytes to enhance leukocyte recruitment as well as the phagocytosis of the accumulated crystals. It was also found that MSU can activate a specific inflammatory cascade leading to the release of interleukins like IL-1. It binds to various signaling protein complexes in leukocytes, ultimately activating Caspase-1 [3-4].

Xanthine Oxidase (XO) has a major role in the uric acid production as XO is responsible for catalyzing the oxidation of hypoxanthine to form xanthine and finally to uric acid. Thus, this enzyme coordinates the reaction and produces uric acid from its precursors [5]. Similarly, certain enzymes such as Cyclooxygenase-2 (COX-2) contributes its role in the gouty inflammation, though it heightened expressions in the presence of the accumulated MSU crystals, which in turn enhances the production of inflammatory prostaglandins leading to the increased production of IL-1β. Thus, COX-2 plays a major role in arousing the inflammatory responses and thus taking part in the advancement of the acute inflammation in the gouty arthritis patients [6].

There are certain measures taken in order to treat the disease by using Non steroidal Anti inflammatory Drugs (NSAIDs), Colchicine or Gluco corticoids. NSAIDs are the class of drugs which causes COX-2 inhibition whereas Colchicine is an antimycotic alkaloid that apart from disturbing the microtubule polymerization, it also inhibits the inflammation by preventing the IL-1β processing which was stimulated by the MSU crystals [7-8]. Gluco corticoids also inhibit the inflammatory cascade through the inhibition of IL-1β [9].

Though, these drugs are being used to prevent the inflammatory arthritis, yet they are found to have negative effect over the other physiological systems. It was found that frequent use of NSAIDs can enhance the risk of gastrointestinal side effects, cardiovascular injury, thrombosis, artherosclerosis and hypertension like problems [10]. On the other hand, Colchicine causes the risk of diarrhea and other gastrointestinal side effects whereas Glucocorticoids show different side effects including hypertension, increased systemic vascular resistance and cardiac contractibility [11-12]. Due to the above mentioned side effects of the drugs used, there is a need of exploring other alternatives which have medicinal properties for treating the disease as well as avoiding the possible side effects.

In this regard, *Jatropha curcas*, has been known to have many medicinal properties such as, antioxidant, anti-inflammatory, antidiabetic, antihelmintic, antidiarroheal as well as antiulcer activities [13-14]. We herein the present study tried to explore computationally the effect of phytochemicals present in the *Jatropha* on the two proteins, Xanthine Oxidase and COX-2.

MATERIALS AND METHODS

Protein structure retrieval and active site predictions

The protein sequence of human Xanthine Oxidase and Cyclooxygenase-2 was retrieved from UniProt database. The

retrieved amino acid sequences were subjected to modeling using Swiss-Model. The predicted models were validated using PROCHECK, ERRAT and VERIFY_3D present at Structural Analysis and Verification server (http: //nihserver. mbi. ucla. edu/SAVES/). Further, the proteins were optimized and energy minimized using Mod Refiner. The active site of both the proteins was predicted using COACH server (http: //zhanglab. ccmb. med. umich. edu/COACH/).

Substrate selection

The 3D structures of phytochemicals from *Jatropha* species were retrieved from Knapsack 3D server using PRODRG server (http: //davapc1. bioch. dundee. ac. uk/prodrg/). The ligand optimization was carried out under MFF94 using Ligand Scout 3.12. The optimized ligands were further docked into the active site of the proteins to estimate their binding affinity. In addition, the three dimensional chemical structure of commercial COX-2 and Xanthine Oxidase inhibitors was also isolated and docked with corresponding protein.

Molecular docking screening

The optimized ligands and commercial inhibitors were docked into the active site of the Xanthine Oxidase and Cyclooxygenase-2 using AutoDock4.2 (MGL Tools) as described by Khursheed *et al* [15-16]. For each ligand, twenty five independent docking runs were carried out following Lamarckian Genetic Algorithm performed for each ligand. Further, protein ligand interactions were evaluated using Lig Plot+.

RESULTS AND DISCUSSION

In the present study, an integrated approach to model human Xanthine Oxidase and Cyclooxygenase-2 & predict the efficacy of phytochemicals from the *Jatropha* species against both the proteins. The Xanthine Oxidase and COX-2 protein structures were modeled based on the alignment with the other proteins using NCBI BLASTp. The analysis demonstrated that human Xanthine oxidase showed a maximum similarity with E803V mutated version of the human Xanthine oxidase (PDB Id: 2E1Q) with sequence identity of 99.92%, GQME score of 0.99 and QMEAN4 score of -1.35. The energy minimization and optimization of the modeled structure using Mod Refiner showed a RMSD of 0.440Å and TM-score of 0.9987. Further validation of optimized protein structure using SAVES demonstrated that 91.5%, 8.4% and 0.1% of amino acid residues in core, allowed and disallowed regions, 87.25% of amino acid residues had an average 3D-1D score > 0.2 (Verify 3D) with an overall quality factor of 81.915 (ERRAT). For COX-2, N580A Prostaglandin G/H synthase 2 from *Mus musculus* demonstrated a maximum sequence identity of 86.26% with GQME score of 0.92 and QMEAN4 score of -0.60. Further energy optimization showed a RMSD of 0.497 Å and TM-score of 0.9965. Validation of the human COX-2 showed that that 92.0%, 7.7% and 0.2% of amino acid residues in core, allowed and disallowed regions, 89.33% of amino acid residues had an average 3D-1D score > 0.2 (Verify 3D) with an overall quality factor of 88.787 (ERRAT) (fig. 1).

The active site of any protein is critical for its activity, thus blocking it with a suitable ligand may result into inhibition of the protein either partially or completely. In this regard, it becomes highly essential to determine the amino acid residues of the protein that forms the active site. The COACH analysis demonstrated that crucial amino acid residues forming the active site of human Xanthine Oxidase include Gln110, Cys148, Gln734, Gly764, Phe765, Gly766, Glu769, Leu840, Arg847, Ala877, Phe878, Arg879, Gly880, Phe881, Thr977, Met1005, Gly1006, Gln1007, Leu1009, Lys1012, Thr1044, Ala1045, Ala1046, Ser1047, Ser1049, Thr1077, Ser1080, Ser1082, Gln1194, Lys1257, Val1259 and Glu1261. Similarly, Met113, Val116, Arg120, Val349, Leu352, Tyr355, Leu359, Leu384, Tyr385, Trp387, Phe518, Met522, Gly526, Ala527, Ser530, Leu531 formed human COX-2 active site residues predicted with a C-score of 0.89 with a cluster size of 190.

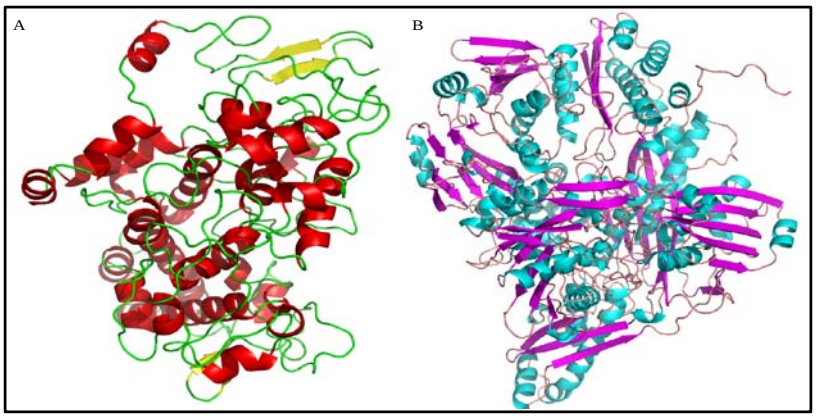

Fig. 1: It shows the Modeled structures of (A): Cyclooxygenase – 2 and (B) Xanthine Oxidase

Exploring the phytochemicals for curing the diseases and disorders had been a crucial strategy for the pharmaceutical development. These phytochemicals may provide a suitable scaffold for the design and development of novel drug with better specificity and efficacy. In this regard, we explored the potential of phytochemicals from *Jatropha* species for the treatment of gout and related inflammations. It is already known that *Jatropha isabellei* has been used in South America folk medicine for the treatment of arthritic diseases particularly gout. In addition, the gout is a form of inflammatory arthritis that results into severe pain, swelling and tenderness. Thus, targeting hyperuricemia and inflammation at the same time is essential in gout disease. It is important to mention that extracts of *Jatropha* species have also demonstrated anti-inflammatory properties. But still, the phytochemicals from *Jatropha* have not been explored completely for treatment of gout and corresponding inflammation. Our analysis demonstrated that Kaempferol 3-alpha-L-arabinopyranoside (KNApSAcK_ID: C00005133) demonstrated the highest binding affinity towards XO (BE_{XO} = -10.34 kcal/mol); while Jatrophenone (KNApSAcK_ID: C00031919) demonstrated the highest binding affinity for COX-2 (BE_{COX2} = -9.43 kcal/mol). Kaempferol 3-alpha-L-arabinopyranoside interacted with Met1038, Phe798, Gln1194, Ser1080, Glu802 and Ser1082 residues of XO via hydrogen bond formation and with Gly797, Gly796, Gln1040, Ala1078, Gly1260, Ala1079, Glu1261, Thr1077, Arg912, Gly799, Phe911, Ala910 and Gln767 residues via hydrophobic interactions. Jatrophenone interacted with Tyr385 of COX-2 via hydrogen bond formation and with Trp387, Phe518, Val523, Met522, Leu352, Ser353, Val116, Tyr355, Leu359, Arg120, Leu531, Val349, Ser530, Gly526, Ala527, Phe381 and Leu384 residues via hydrophobic interactions. The binding and interaction analysis of the selected phytochemical docked with XO and COX-2 has been demonstrated in table 1 and table 2 respectively. A critical analysis of the binding pattern demonstrated that Jatrophone (KNApSAcK_ID: C00003446), 6β-hydroxy-4-stigmasten-3-one (KNApSAcK_ID: C00029573) and Palmarumycin CP1 (KNApSAcK_ID: C00035859) had a good affinity for both, XO and COX-2 ((fig. 2).

Table 1: It shows the binding and interaction analysis of the selected phytochemical docked with XO using Autodock.

KNApSAcK_ID	Binding Energy (Kcal/mol)	Estimated inhibition constant (in µM)	Interaction Analysis	
			Hydrogen Bonds	Hydrophobic Bonds
C00002984	-8.38	0.71664	Arg912, Gly1260, Gln1194, Ser1082, Ala1079	Phe914, Glu1926, Phe798, Ser1080, Gly799, Ala1078, Val108, Thr1083, Gln1040, Lys1045, Ile1190
C00003445	-8.58	0.51398	Gln1040	Gln767, Ala1079, Gly799, Glu1261, Arg912, Phe798, Gly1260, Gln1194, Met1038, Ala1078, Ser1082, Ser1080
C00003446	-9.18	0.18652	Gln1040	Thr1083, Gly1260, Ser1082, Ser1080, Glu1261, Thr1077, Arg912, Ala1078, Ala1079, Gln767, Met1038, Gln1194
C00003672	-9.64	0.08618	Phe911	Asp1084, Lys1257, Ala1258, Ser1080, Val1259, Ser1082, Gln767, Glu1261, Ala1078, Ala1079, Arg912, Gly913, Phe914, Ala910, Phe798, Gly799, Gln1194, Thr1077, Gln1040, Gly1260, Met1038, Thr1083, Lys1045
C00005133	-10.94	0.00963	Met1038, Phe798, Gln1194, Ser1080, Glu802, Ser1082	Gly797, Gly796, Gln1040, Ala1078, Gly1260, Ala1079, Glu1261, Thr1077, Arg912, Gly799, Phe911, Ala910, Gln767
C00029573	-8.61	0.48806	Gln1194	Lys1257, Ala1258, Thr1081, Ser1082, Met1038, Ser1080, Arg912, Phe911, Thr1077, Gln767, Ala1079, Ala1078, Glu802, Gly913, Ala910, Phe914, Glu1261, Phe790, Gly799, Gln1040, Gly1260, Val1259, Lys1045
C00031852	-8.58	0.5118	Gln1194	Ala910, Ala1079, Glu1261, Ser1080, Met1038, Gln1040, Gly1260, Arg912, Phe798, Phe914, Phe911, Gly799
C00035859	-8.73	0.4017	Thr1083, Ser1082, Ser1080	Gly1260, Gly1261, Met1038, Phe798, Arg912, Gln1040, Val1259, Gln1194, Lys1045, Val1081, Ala1258
C00039286	-9.62	0.08934	Gly913, Gly1260, Thr1083	Phe798, Glu1261, Thr1077, Val1259, Ser1080, Ser1082, Gln1040, Gln1194, Met1038, Gly799, Arg912, Phe914, Gln767, Ala1078, Glu802, Phe911, Ala910
C00047630	-8.64	0.4607	Arg912	Gly799, Glu1261, Phe798, Ala1079, Ala1078, Thr1077, Gln1040, Ser1080, Met1038, Ser1082, Gly1260, Gln1194

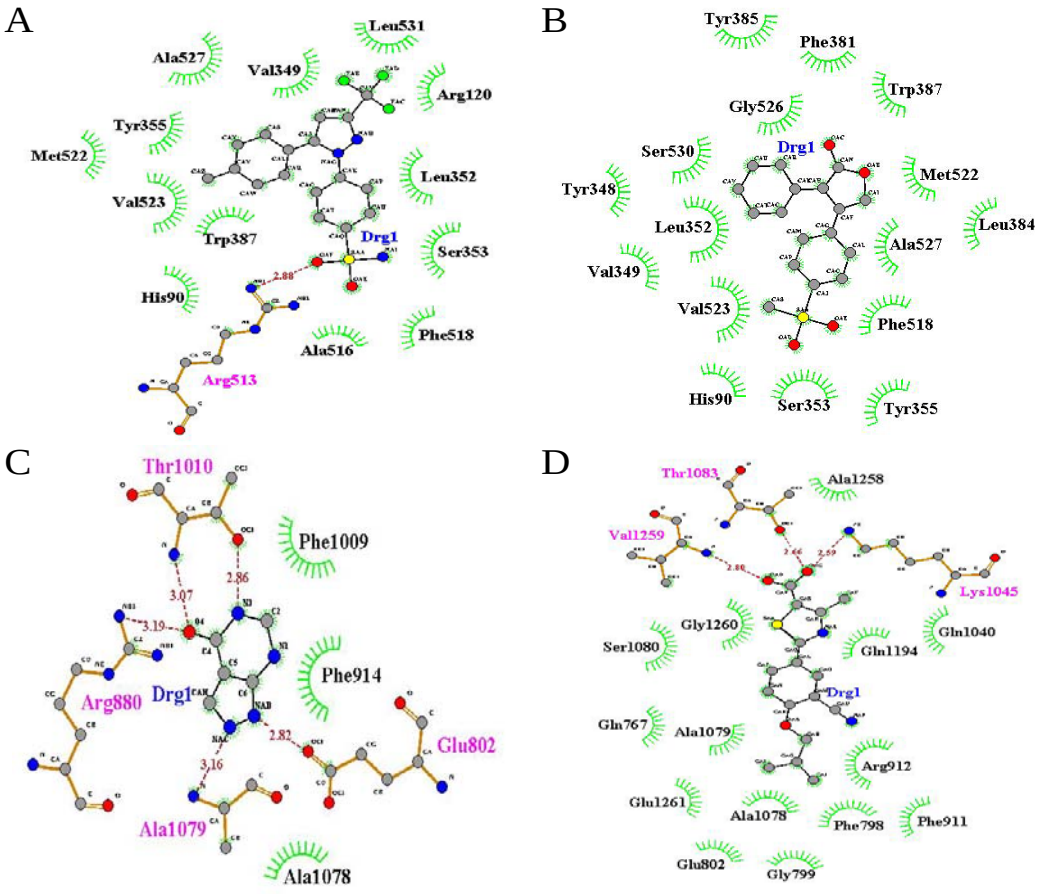

Fig. 2: It shows the Hydrogen and Hydrophobic interaction analysis of COX – 2 (A, B) and XO (C, D)

Table 2: It shows the binding and interaction analysis of the selected phytochemical docked with COX - 2 using Autodock

KNApSAcK_ID	Binding Energy (Kcal/mol)	Estimated inhibition constant (in µM)	Interaction Analysis	
			Hydrogen Bonds	Hydrophobic Bonds
C00003446	-8.7	0.41778	0	Met522, Gly526, Leu384, Trp387, Tyr385, Phe518, Phe381, Ser530, Val349, Val523, Ala527, Leu352, Tyr348
C00003484	-8.48	0.60489	0	Tyr355, Val349, Leu359, Ser353, Leu352, Arg120, Met522, Val523, Phe518, Ser530, Trp387, Ala527
C00029573	-8.79	0.35794	Leu93	Ile92, Val189, Val116, Tyr115, Arg120, Lys83, Ser119, Leu123, Glu524, Phe470, Tyr122, Ser471
C00031919	-9.43	0.1219	Tyr385	Trp387, Phe518, Val523, Met522, Leu352, Ser353, Val116, Tyr355, Leu359, Arg120, Leu531, Val349, Ser530, Gly526, Ala527, Phe381, Leu384
C00035859	-9.59	0.09415	His90, Tyr355	Ser353, Arg513, Val523, Val349, Tyr348, Ser530, Tyr385, Leu352, Gly526, Ala527, Phe518
C00038406	-8.42	0.67057	0	Leu384, Trp387, Tyr385, Gly526, Phe518, Phe381, Val523, Leu352, Met522, Arg120, Ala527, Ser530, Val349, Leu359, Ser353, Ile345, Tyr355, Val116, Leu531, Met113, Leu117
C00047940	-8.55	0.5441	Tyr355	Met522, Trp385, Gly526, Leu352, Phe518, Val523, Ser530, Ala527, Leu531, Val349, Val116, Arg120

CONCLUSION

A significant amount of research has already been done for the development of anti-gout agents, which has considerably reduced its cases. However, there still exists an urge to explore new ligands that could be used for the purpose. In the present investigation, we, through *in silico* approach demonstrated the possible anti-gout and anti-inflammatory effect of phytochemicals from *Jatropha* species by inhibition of Xanthine Oxidase and Cyclooxygenase-2. The predicted ligands could provide a scaffold for the development of novel leads with better affinity and specificity. Further, *in vitro* and *in vitro* validation need to be done.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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