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Assessment of Anti-arthritis Efficacy of *Moringa oleifera: In-silico* Molecular Docking

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Abstract: ***Background:*** The severity of rheumatoid arthritis (RA), a chronic, inflammatory, and systemic autoimmune disease, on a patient's joints varies. Age, gender, genetics, and environmental exposure (tobacco use, exposure to air pollution, and occupational exposure) are risk factors. If left untreated, Felty syndrome can develop into a variety of consequences, including rheumatoid vasculitis, persistent joint damage needing arthroplasty, and Felty syndrome necessitating splenectomy. The objectives of treatment for RA are to alleviate discomfort and prevent/slow additional damage because there is no known cure. Munga, also known as Moringa oleifera Lam., is one of the most significant plants grown extensively in India. This plant, Moringa oleifera Lam, is used extensively as a dietary supplement and has valuable pharmacological properties including anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-cancer, antimicrobial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic action. This plant has great therapeutic properties in every area. It is a good source of milk protein, vitamin A, and vitamin C. Alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides, fixed oil, and lipids are only a few examples of the several active phytoconstituents that are present. ***Aim and Objective:*** The goal of the current study was to evaluate *M. oleifera* leaf anti-arthritic efficacy. ***Method:*** *In -silico* molecular modelling studies for assessment of anti-arthritic potential of *M.oleifera* leaf was designed taking quercetin and niazirinin as lead molecule found in the ethanolic leaf extract(as per literature survey) against ***HK-2*** protein. A grid-based docking strategy was used to determine the binding using the Auto Dock software. Merck Molecular Force Field was used to build the 2D structures of compounds, convert them to 3D, and then energetically reduce them up to arms gradient of 0.01. (MMFF). ***Result:*** The finding of molecular modelling of lead molecule with ***HK-2*** protein showed that both the selected molecules have good affinity towards ***HK-2*** protein but quercetin found to be high binding affinity. The binding energy of quercetin and niazirinin against ***HK-2*** protein was found to be -6.66 & -5.58 Kcalmol^{-1} respectively.

Keywords: RA, quercetin, niazirinin and ***HK-2*** protein.

Research Paper

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INTRODUCTION

Rheumatoid arthritis is a systemic illness that can appear as rheumatoid nodules, vasculitis, ocular inflammation, and cardio pulmonary disease. There is no genetic component to rheumatoid arthritis. According to researchers, some people have genes that predispose them to the illness [1]. Rheumatoid arthritis does not necessarily occur in those who carry these genes. The genes are often activated by a "trigger," such as an infection or environmental influence. The immune system reacts incorrectly when the body is exposed to this trigger. The immune system starts to make molecules that fight the joint rather than protecting it. This is what could cause rheumatoid arthritis to appear. It is an autoimmune illness, which means that healthy tissues are wrongly attacked by the body's immune system [2]. In healthy joints, the lining is very thin and contains few blood vessels, but in joints affected by rheumatoid arthritis, the lining is thick and densely packed with white blood cells. Interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-alpha), two chemicals secreted by white blood cells, cause discomfort, joint swelling, and joint degeneration [3]. The existence of new cytokines like IL-17 and IL-18 was recently discovered. These cytokines trigger the secretion of proteoglycan- and collagen-degrading enzymes by synovial fibroblasts and chondrocytes in the surrounding articular cartilage, which results in tissue degradation and the production of RANK ligand

(RANKL), a factor in the aetiology of chronic arthritis. The synovial begins to multiply and spread because to the production of numerous cytokines and mediators of inflammation; this is known as pannus. The following stage, fibrosis, results in a lack of joint motion and is referred to as ankylosis [4-6].

Moringa oleifera is also known as horse radish tree and drum stick tree. Munga plants provide large and rare combination of zeatin, quercetin, beta – sitosterol, kaemopferol, and caffeoylguinic acid. Vital minerals present in the Moringa oleifera include iron, potassium, calcium, copper, zinc, magnesium, manganese etc. The main constituents of Moringa plant are: deic, palmitic and stearic acid, saponins, glycoside, gum, protein Vitamins: A (8855 IU per 100g), B1, B2, B3, C Minerals: calcium, iron, phosphorus, magnesium The leaves, flowers and pods are used as significant sources of vitamins A, B and C, riboflavin, nicotinic acid, folic acid, pyridoxine, beta-carotene, calcium, iron, and alpha-tocopherol. Because of its high flavonoid content, the *Moringa* genus has a strong antioxidant activity. The flavanol and glycoside forms of flavonoids are found in the majority of the flavonoids in the genus. Rutin, quercetin, rhamnetin, kaempferol, apigenin, and myricetin are the most frequent flavonoids in the genus. The majority rich glucosinolate present in the species is 4-O-(α-L-rhamnopyranosyloxy)-benzyl glucosinolate. Gallic acid is the most abundant phenolic acid in M. oleifera leaves. The leaves also contain elagic acid ferulic acid, caffeic acid, o-coumaric acid and chlorogenic acid. β-sitosterol was isolated from the leaves and seeds. Plant showed Antispasmodic, Antihypertension, Anti-inflammation, Antifertility, Antihyperglycemic, Antihyperlipidemic, and Hypocholesterolemic, Antiviral, Antileishmanial, Anti-convulsant, Anti-microbial & anticancer activity [7-9].

Experimental works

Molecular docking studies Quercetin and Niazirinin against *HK-2* Protein

Ligand Preparation:

2D Structure of ligands like quercetin, and niazirinin were drawn using ChemSketch [10], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

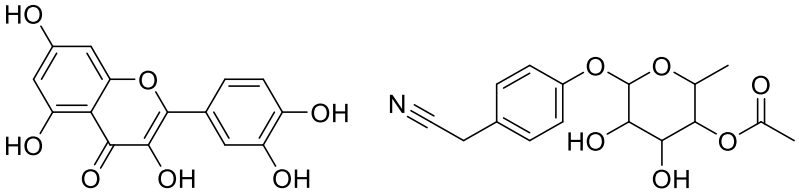


Figure 1: 2D structure of quercetin and niazirinin

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points is given in table 1 [11-12].

Table 1: Grid parameters used in current docking analysis of HK-2.

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	HK-2	40	40	40	0.414	8.065	4.076	17.906

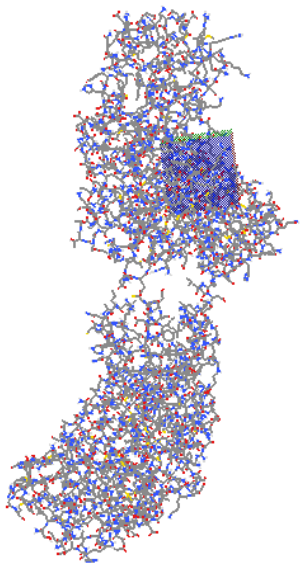


Figure 2: Grid box covering all active sites in HK-2 receptor

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [13].

Docking Study

Crystal structure

The crystal structure of the protein consisting of HK-2 receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (1n45.pdb) registered in the Protein data bank was used [14]. The complex ligand was separated by using Chimera software.

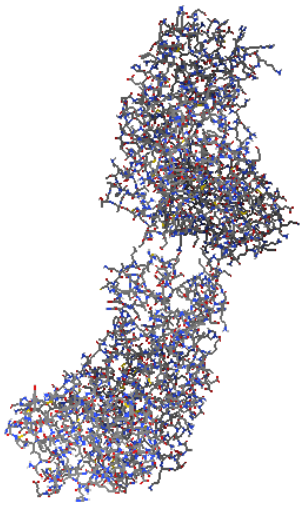


Figure 3: Crystal structure of HK-2 receptor (PDB ID-1n45)

Processing of Protein

The downloaded receptor protein is having two chains, i.e. chain A, and B. Out of these two chains, chain A was selected for experimental purpose and other chain B was removed from it. The bound ligand Heme was separated from the macromolecular complex by using software Chimera [15].

Molecular Docking Simulation Studies

Docking of ligands like quercetin and niazirinin against human HK-2 receptor was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [15].

Toxicity & ADME-T Studies

The ligand molecules viz. quercetin and niazirinin were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [16-18].

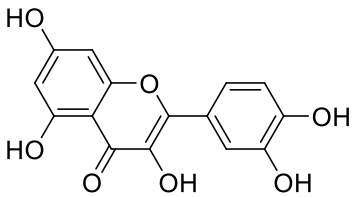
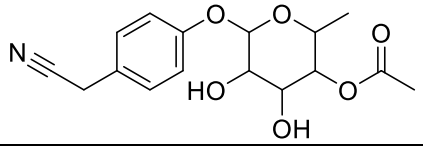
RESULT AND DISCUSSION

In -silico molecular modelling studies for assessment of anti-arthritic potential of *M.oleifera* leaf was designed taking quercetin and niazirinin as lead molecule found in the ethanolic leaf extract(as per literature survey) against ***HK-2*** protein. Anti-arthritic activity was assessed by computational based molecular docking study. As per literature survey ethanolic leaf extract of *Moringa oleifera* contained quercetin and niazinin, so these compounds taken in consideration for docking against ***HK-2*** protein.

HK2 is highly upregulated in activated T cells and plays important roles in B cell lymphoma cell apoptosis. In human monocyte-derived DCs, Toll-like receptor (TLR)-4-dependent upregulation of glycolysis leads to enhanced HK2 activity involving p38-MAPK-dependent hypoxia inducible factor-1(HIF-1) accumulation. Besides, phosphoinositide-3-kinase (PI3K)/AKT pathways are involved in the phosphorylation of rate-limiting mitochondrial HK2. Janus kinase/Signal transducer and activators of transcription (JAK/STAT) signaling was also revealed to mediate glucose uptake and HK2 expression. Zhou KL *et al.*, 2021 observation suggested that aberrant expression of HK2 may associate with pathological changes in RA by mediating related signalling pathways. More surprisingly, HK2 antagonists, including ablation of glycolytic genes or treatment with 3-bromopyruvate, significantly relieved the severity of several arthritis models. Targeting a specific intracellular compartment of HK2 (i.e., nucleus, cytosol, or mitochondria) will provide a selective way to block the harmful effect of the enzyme in RA without affecting the glucose metabolism of normal cells [17]. Therefore, HK2 is an attractive and selective target for the treatment of arthritis and is safer than global glucose metabolism inhibition.

The finding of molecular modelling of lead molecule with ***HK-2*** protein showed that both the selected molecules have good affinity towards ***HK-2*** protein but quercetin found to be high binding affinity. The binding energy of quercetin and niazirinin against ***HK-2*** protein was found to be -6.66 & -5.58 Kcalmol^{-1} respectively (table 2). The binding mode showed in fig.4-5 The docking interaction result revealed that the quercetin showed conventional hydrogen binding at ASP A:209, GLY A:231, SER A:449, ASP A:413, THR A:232, THR A;188, LYS A:418, GLU A: 446 and Pi-sigma binding at ASP A:84 having ASP A: 417 covalently. The 2D binding interaction showed in fig.6-7 & 3D binding interaction displayed in fig.8-11. The pharmacokinetic profile of quercetin & naizirnin reveals that it is having good pharmacokinetic profile but with the presence of any major toxic effects including mutagenicity, tumorogenicity and reproductive effects. The pharmacokinetic and toxicity profiling results of ligands like quercetin and niazirinin were shown in figure 12-13.

Table 2: Results of docking of ligands like quercetin and niazirinin against human HK-2 receptor

Sl. No	Compound Name	Structure	Binding Energy (Kcal/mole)
1	**Quercetin**		-6.66
2	**Niazirinin**		-5.58

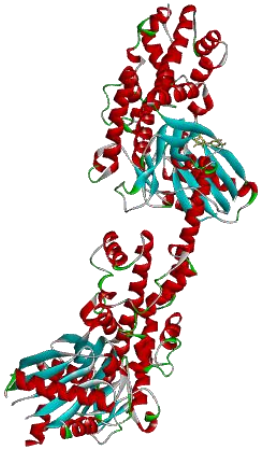


Figure 4: Binding mode of quercetin within the active site of human HK-2 receptor

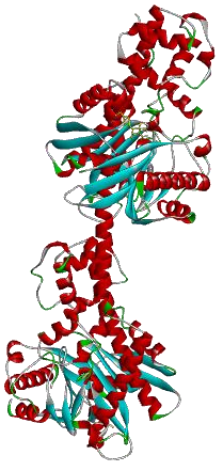


Figure 5: Binding mode of niazirinin within the active site of human HK-2 receptor

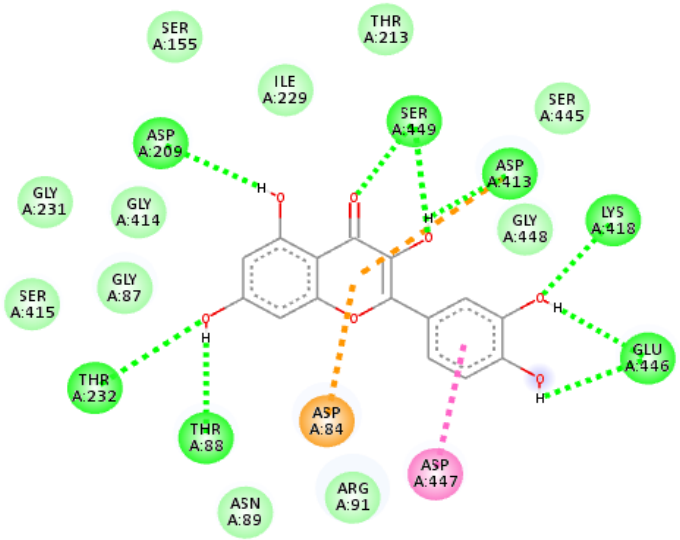


Figure 6: Two-dimensional binding mode of quercetin within the active site of human HK-2 receptor

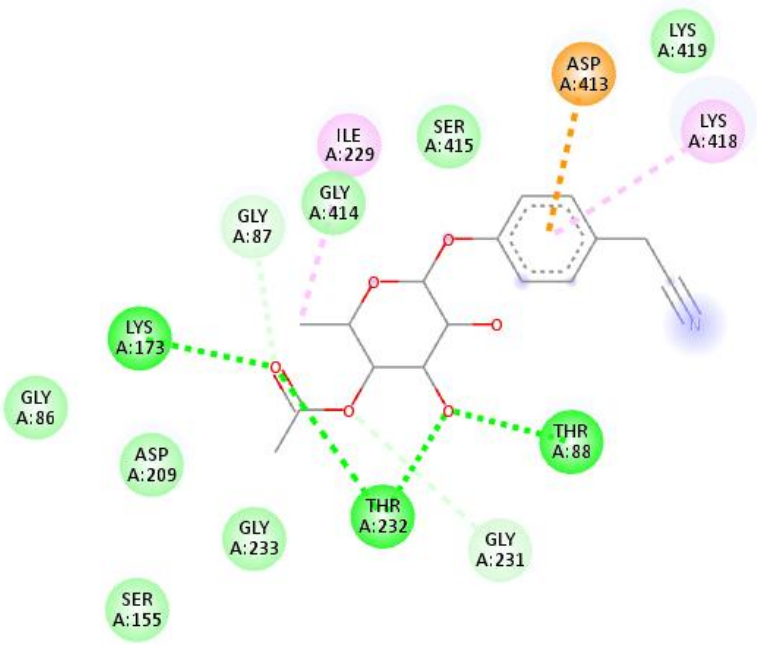


Figure 7: Two-dimensional binding mode of niazirinin within the active site of human HK-2 receptor

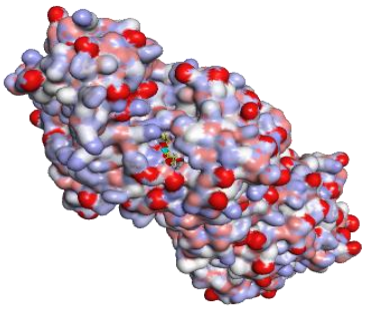


Figure 8: Three-dimensional binding conformation of quercetin within the active site of human HK-2 receptor

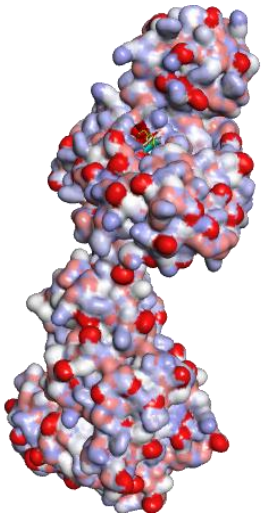


Figure 9: Three-dimensional binding conformation of niazirinin within the active site of human HK-2 receptor

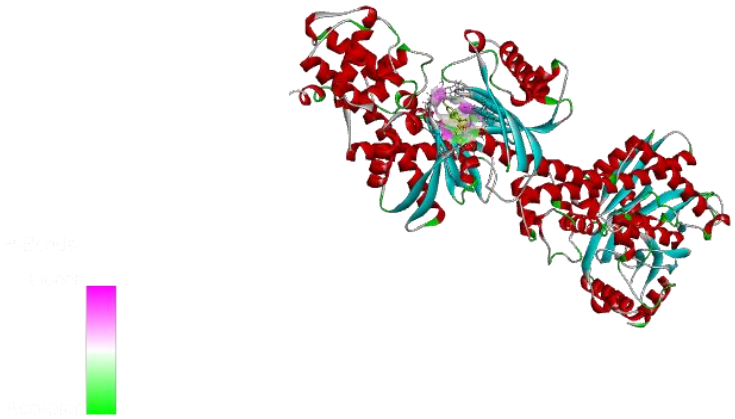


Figure 10: Three-dimensional binding mode of quercetin within the active site of human HK-2 receptor

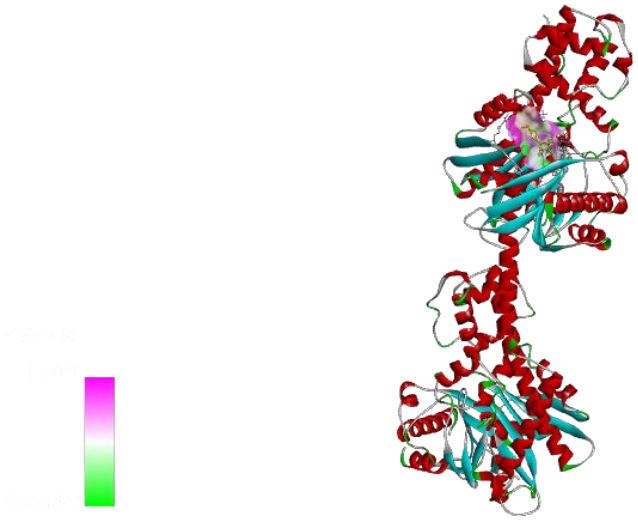


Figure 11: Three-dimensional binding mode of niazirinin within the active site of human HK-2 receptor

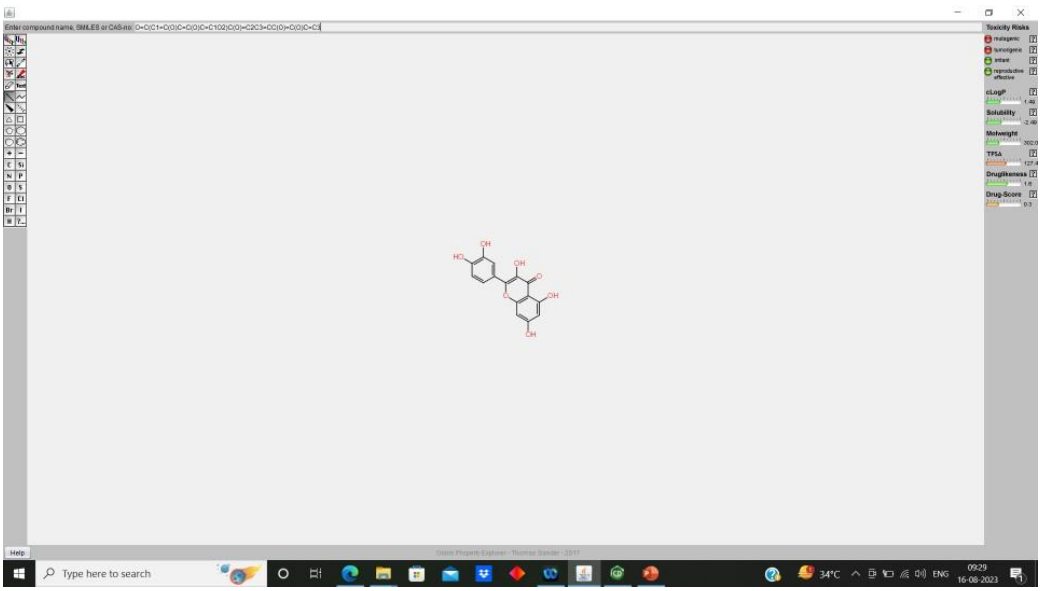


Figure 12: Pharmacokinetic and toxicity profiling of Quercetin

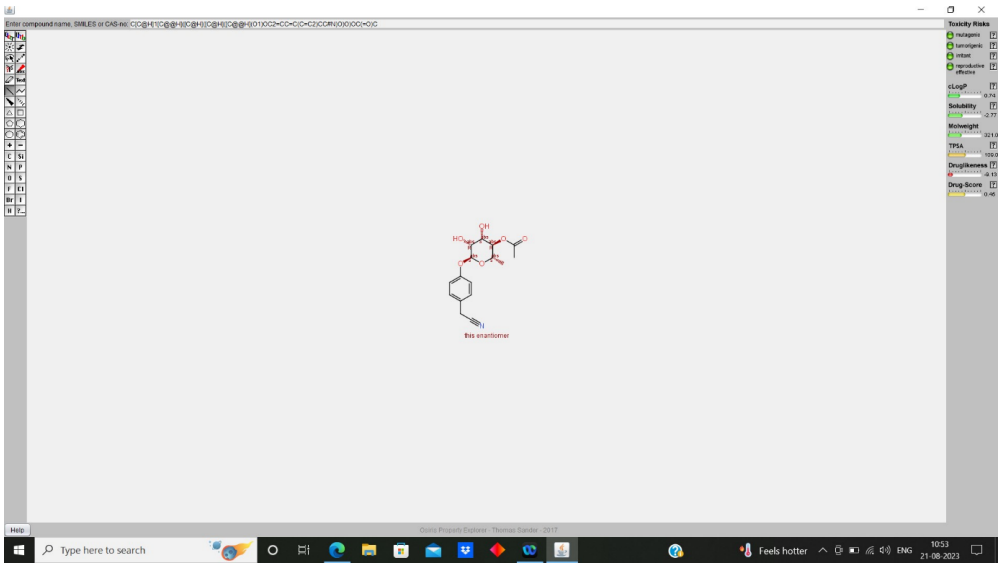
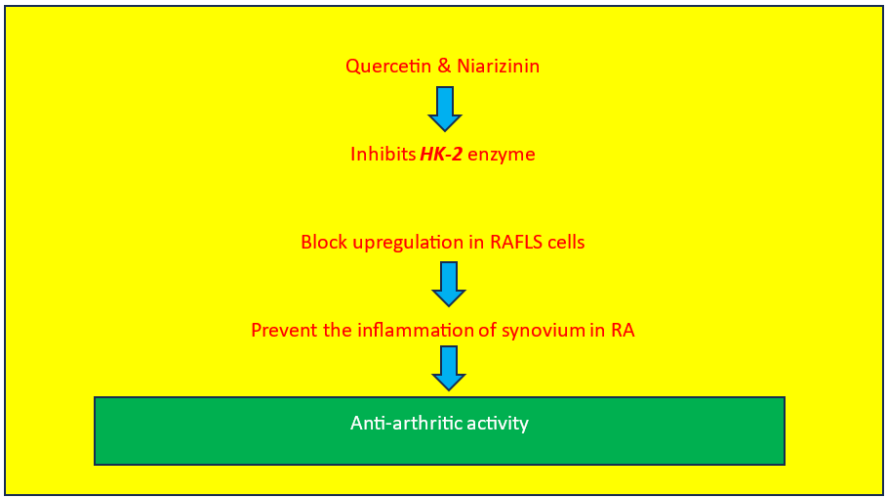
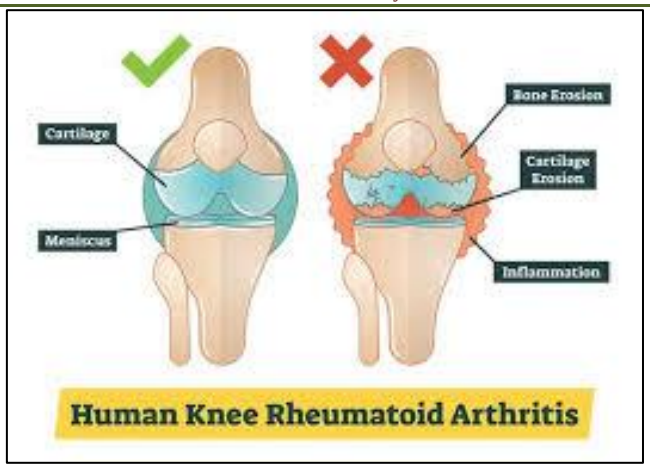


Figure 13: Pharmacokinetic and toxicity profiling of Niazirinin

Divulgence of Investigation

The results of the current study demonstrated that M. olegifera's ethanolic leaf extract contains flavonoids and phenolic glycosides. According to a literature review, leaves included quercetin and niarizinin, which were chosen as the primary compounds for a computationally based prediction analysis to evaluate their effectiveness in treating arthritis. The results of the current study revealed that Niarizinin has inhibitory effect on ***HK-2*** protein whereas quercetin is a powerful ***HK-2*** protein antagonist.





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