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Modeling and In-Silico Evaluation of Some Flavonoids as Cyclooxygenases Inhibitors Phytochemicalsognes

Haifaa Rasheed Muhsin
Branch of pharmacognesy, College of pharmacy, Kerbala University, Iraq

Original Research Article

***Corresponding author**
Haifaa Rasheed Muhsin

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Abstract: In the inflammation process, the enzyme cyclooxygenase-2 expressed and its function is prostaglandins synthesis of from arachidonic acid so that to inhibit inflammation proses, COX-2 must be inhibited. In current research a twenty-six flavonoids compounds included flavonols (n= 10), flavones (n= 7) and anthocyanins (n= 9) were studied to predict their affinity to attach to the active site of both COX-1 and COX-2 enzymes and to predict the selectivity of each compound to COX-2, this done using docking method. Docking simulation for each compound done using 1-click docking tool. Some of the studied compound (n= 13) had strong predicted affinity to COX-2 combined with strong affinity to COX-1, so that they are non-selective inhibitors, additionally, an exception was found for Rosinidin and Pulchellidin which tends to bind to COX-2 with stronger affinity than COX-1, as (-8.1, -7.6,) and (-6.5, -5.2, -2.8) respectively.
Keywords: inflammation, cyclooxygenase-2, flavonoids , Pulchellidin, Rosinidin.

INTRODUCTION

The plants derived natural compounds, the flavonoids are known to be the phenolic compounds extracted from numerous plants around the globe [1]; their effects included antioxidants, antimicrobials, and photoreceptors. The multi-functionality is one of the flavonoids characteristics, and the most recognized is the antioxidant activity, this function happened due to the ability to reduce free radical genesis and to clean out free radicals. The efficacy of flavonoids antioxidants activity in vitro has been the demonstrated in several investigations in the recent years [2].

The process of inflammation is increasingly involved in the development of several diseases. Additionally, treatments for chronic inflammatory disorders has not been solved yet. And an urgent need to develop a new anti-inflammatory materials characterized by relative safety [3]. On the other hand, this type of compounds has existed in the food that demonstrated several advantageous effects on health. Furthermore, the anti-inflammatory features of some flavonoids have been investigated, in order to find and determine their potential usefulness as therapeutic compounds for the inflammation management [4]. Additionally, there are various pathways of work have been suggested to spot light on the in-vivo flavonoid anti-inflammatory property, like the alteration of the production of pro-inflammatory compounds. [5].

Furthermore, many enzymes like the cyclooxygenase, xanthine oxidase, phosphodiesterase, Ca(+2)-ATPase, lipoxygenase, and others, found to possess an anti-inflammatory activity in inflammation [6]. From all mentioned above screening of flavonoids as natural compounds for their anti-inflammatory activity and the proposed mode of action is considered an important subject. In current work, this done for some flavonoids (flavonols, flavones, and anthocyanins) using in silico approach.

MATERIALS AND METHODS

Three dimensional structure comparison and pairwise sequence alignment

The cyclooxygenase enzymes COX-2 (PDB ID: 5kir) [7]. and COX-1 (PDB ID: 3n8x) [8] were downloaded from protein databank database. The proteins pairwise sequence alignment and structure comparison between COX-2 and COX-1 done and its root means square deviation RMSD was calculated through UCSF chimera tool [9].

Protein preparation

The COX-2 and COX-1 molecules prepared for docking through removing water molecules, ligands, and other hetero atoms from the protein three dimensional; by UCSF Chimera tool [9].

Ligand preparation

Flavonoids Inchi key formulae were taken from pubchem database [10]. And their physiochemical

properties were calculated using Mcule property calculator [11].

Docking simulation

Molecular dockings were done using 1-click docking tool [11], using the active site composed of the following amino acids (Arg120, Ser350, Trp387, Tyr385, Leu503, Leu384, Val434, Val523, Arg513, Glu524) for COX-2 and (Arg120, Ser350, Trp387, Tyr385, Leu384, Ile434, Ile523,His513, Glu524) for COX-1 [12]. The COX-2 (PDB ID: 5kir) active site docked using the binding center x= 32.87 , y= 3.848, z= 59.704., and COX-1 (PDB ID: 3n8x) with binding center x= -23.471, y= -52.516, z= 6.618.

RESULTS

The results of both COX-1 and COX-2 structural alignment shown in Figure 1, and proteins sequence alignment and active sites differences were shown in figure 2.

Additionally, the molecular dockings of both cyclooxygenases isoforms listed in tables 1, 2 and 3. And table 4 summarizes flavonoids with strong affinities against COX-2. On the other hand, Table 5 listed physiochemical properties of flavonoids with strong affinity to COX-2 in combination to their affinity to COX-1.

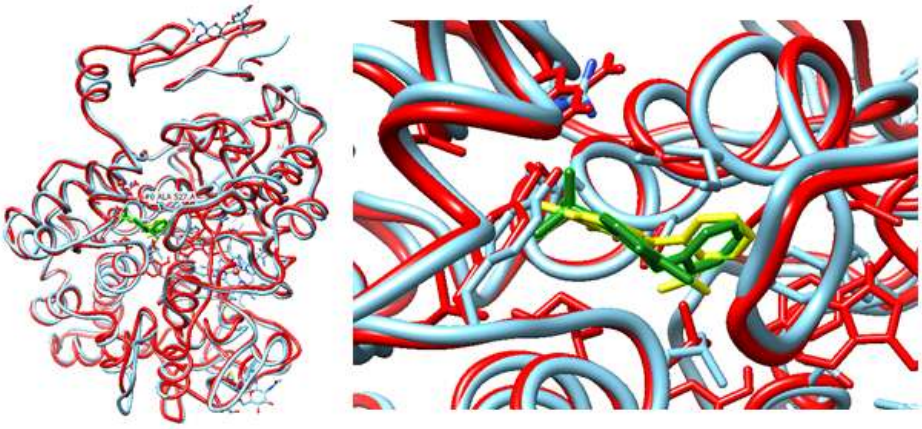


Fig-1: Superposition of COX-2 (red) and COX-1 (light blue) molecules, in complex with mefenamic acid (yellow) and nimesulide respectively. Evaluating superposition's across all 550 amino acids fully populated columns in the final alignment with RMSD of (COX-2) with (COX-1) equals = 0.892, SDM (cutoff 5.0): 17.723, -score: 0.912.

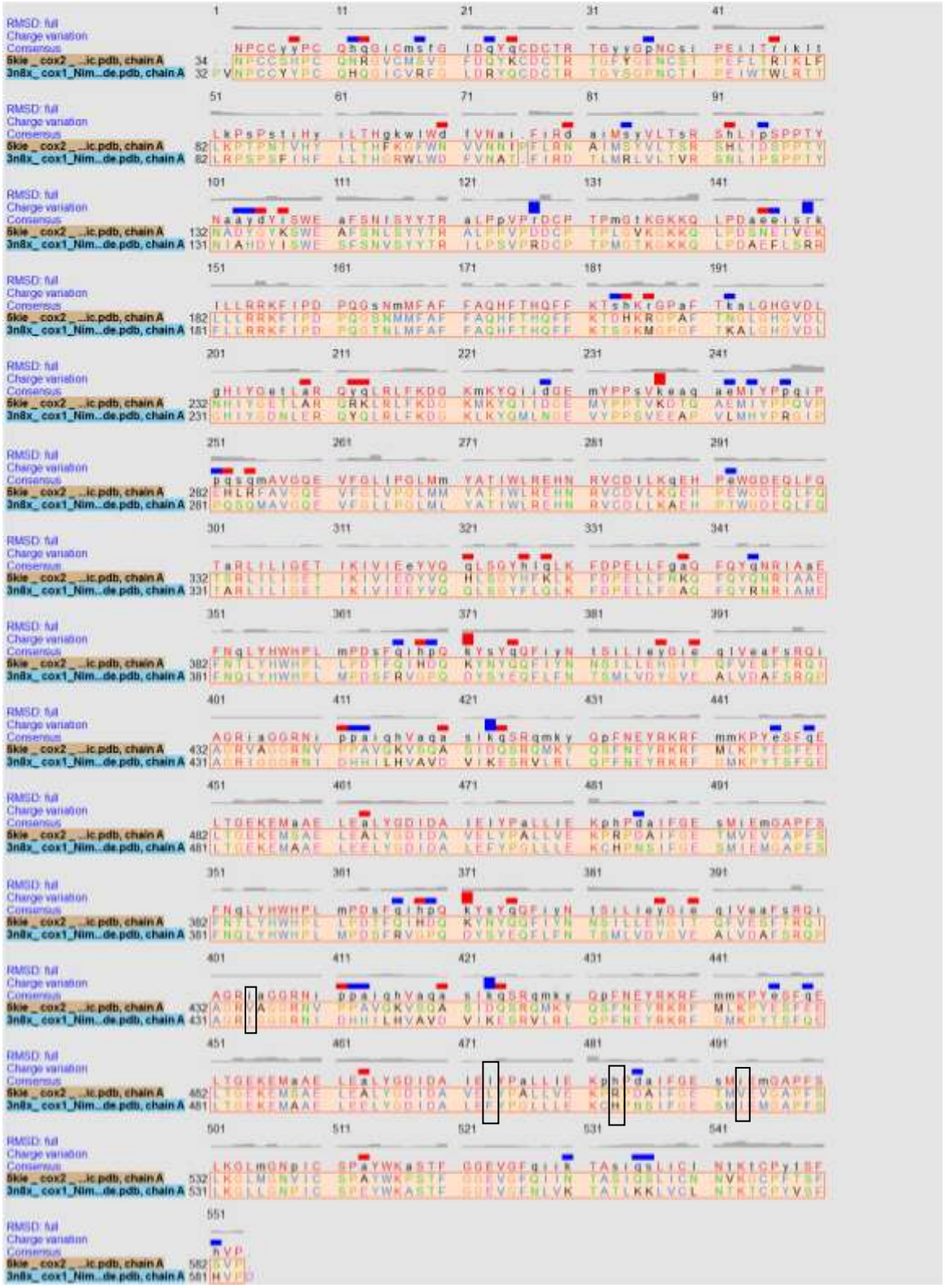


Fig-2: Pairwise global alignment between tow cyclooxygenase enzymes the COX-1 and COX-2 with protein databank ID: 3n8x and 5kie respectively. Boxes indicate substitution of some amino acids within the active site of both cyclooxygenase molecules (Leu503, Val434, Val523, and Arg513), while the other components of the active site (Arg120, Ser350, Trp387, Tyr385, Leu384, and Glu524) still unchanged.

Table-1: cyclooxygenase-2 (COX-2) docking score of studied flavonols with their Inchi key identifiers and structures in comparison to Mefenamic Acid and Diclofenac

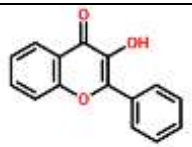
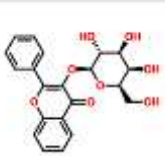
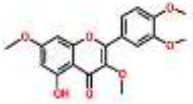
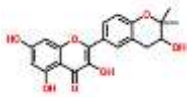
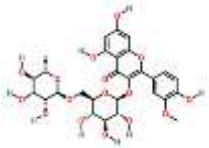
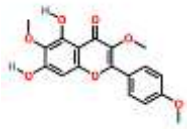
Flavonols	Inchi key	Docking score	Ligand structure	Flavonols	Inchi key	Docking score	Ligand structure
Mefenamic Acid	HYYBABOKPJLUIN-UHFFFAOYSA-N	-8.6		Diclofenac	DCOPUUMXTXDBNB-UHFFFAOYSA-N	-8.6	
3-Hydroxyflavone	HVQAJTFOCKOKIN-UHFFFAOYSA-N	-8.7		Flavonol 3-O-rutinoside	FFIUTTCXMBJITR-WZQYKBDNSA-N	-3.1	
Flavonol 3-O-D-galactoside	XUDNWQSXPROHLK-RCHULGBISA-N	-7.3		Flavonol 3-O-D-glucoside	XUDNWQSXPROHLK-OACYRQNASA-N	-8.1	
Retusin	HHGPYJLEJGNWJA-UHFFFAOYSA-N	-5.8		Glycyrrhiza Flavonol A	UFWHTSBKDGUFOX-UHFFFAOYSA-N	-6.6	
Narcissoside	UIDGLYUNOUKLBM-GEBJFKNCSA-N	3.1		Santin	DWZAJFZEYZIHPO-UHFFFAOYSA-N	-4.5	

Table-2: cyclooxygenase-2 (COX-2) Docking score of studied flavones with their Inchi key identifiers

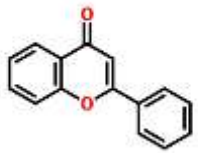
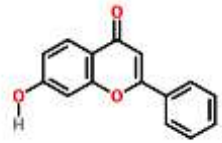
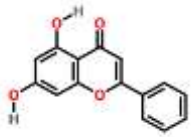
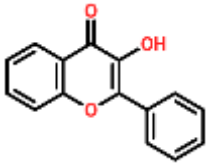
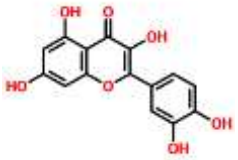
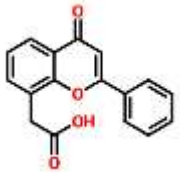
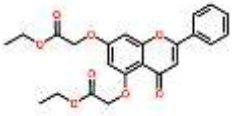
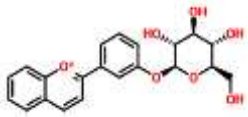
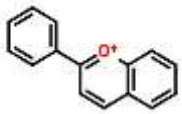
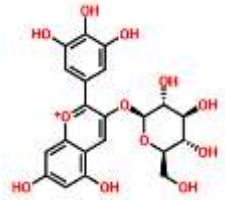
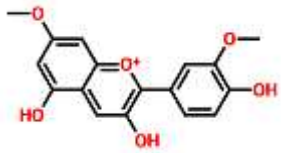
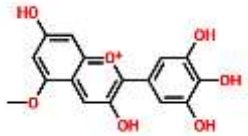
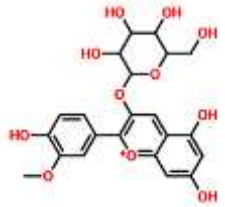
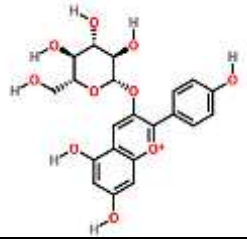
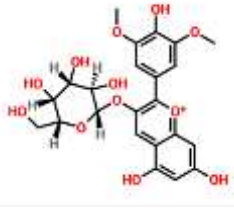
Flavones	Inchi key	Docking sore	Ligand structure	Flavones	Inchi key	Docking sore	Ligand structure
FLAVONE	VHBFFQKBGNRLFZ-UHFFFAOYSA-N	**-9.4**		7-Hydroxyflavone	MQGPSCMMNJKMHQ-UHFFFAOYSA-N	**-9.3**	
Chrysin	RTIXKCRFFJGDFG-UHFFFAOYSA-N	**-8.9**		3-Hydroxyflavone	HVQAJTFOCKOKIN-UHFFFAOYSA-N	**-8.7**	
Quercetin	REFJWTPEDVJJIY-UHFFFAOYSA-N	**-7.9**		Mitoflaxone	TZZNWMJZDWYJAZ-UHFFFAOYSA-N	**-7.6**	
Diethyl flavone-5,7-dioxyacetate	ITJJTERMXJOUEE-UHFFFAOYSA-N	**-5.6**					

Table-3: cyclooxygenase-2 (COX-2) docking score of studied anthocyanins with their Iinchi key identifiers

Anthocyanin	Inchi key	Docking score	Ligand structure	Anthocyanin	Inchi key	Docking score	Ligand structure
Anthocyanin 3'-O-beta-D-glucoside	LVLDBAMRQAHLTF-YMQHIKHWSA-N	-7.4		Flavylium	NWKFECICNXDNOQ-UHFFFAOYSA-N	-9.0	
Delphinidin 3-glucoside	XENHPQQLDPAYIJ-PEVLUNPASA-O	-4.7		Rosinidin	GNONHFYAESLOCB-UHFFFAOYSA-O	-7.3	
Pulchellidin	SVUQABVHSHQZHD-UHFFFAOYSA-O	-7.6		Peonidin-3-glucoside	ZZWPMFROUHHAKY-OUUKCGNVSA-O	-6.7	
Pelargonidin 3-glucoside	ABVCUBUIXWJYSE-GQUPQBGVSA-O	-6.5		Primulin	PXUQTDZNOHRWLI-XSEKTIEYSA-O	-3.2	

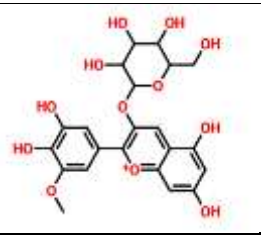
Petunidin 3-glucoside	CCQDWIRWKWIUKK-QKYBYQKWSA-O	-4.2	

Table-4: top docking score of studied flavonoid molecules (higher binding affinity to COX2 than mefenamic acid and diclofenac)

No.	Compound	Inchi key	No.	Compound	Inchi key
1	3-Hydroxyflavone	HVQAJTFOCKOKIN-UHFFFAOYSA-N	4	Flavonol 3-O-D-galactoside	XUDNWQSXPROHLK-RCHULGBISA-N
3	Flavonol 3-O-D-glucoside	XUDNWQSXPROHLK-OACYRQNASA-N	6	FLAVONE	VHBFFQKBGNRLFZ-UHFFFAOYSA-N
5	7-Hydroxyflavone	MQGPSCMMNJKMHQ-UHFFFAOYSA-N	8	Chrysin	RTIXKCRFFJGDFG-UHFFFAOYSA-N
7	Pulchellidin 1.	SVUQABVHSHQZHD-UHFFFAOYSA-O	10	Mitoflaxone	TZZNWMJZDWYJAZ-UHFFFAOYSA-N
9	Quercetin	REFJWTPEDVJJIY-UHFFFAOYSA-N	12	Anthocyanin 3'-O-beta-D-glucoside	LVLDBAMRQAHLTF-YMQHIKHWSA-N
11	2. Rosinidin 3.	GNONHFYAESLOCB-UHFFFAOYSA-O	14	Flavylium	NWKFECICNXDNOQ-UHFFFAOYSA-N

Table-5: structure and molecular dockings of two anti-inflammatory drugs

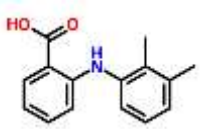
Drug	Inchi key	Docking score	Ligand structure	Drug	Inchi key	Docking score	Ligand structure
4. Mefenamic Acid	HYYBABOKPJLUIN-UHFFFAOYSA-N	-8.6		5. Diclofenac 6.	DCOPUUMXTXDBNB-UHFFFAOYSA-N	-8.6	

Table-6: studied ligands (flavonoids) chemical properties, cyclooxygenase docking scores of in addition to their Lipinski's role of five and role of three, highlighted values fall within optimum range

Property	Mefenamic acid	Diclofenac	3-Hydroxyflavone	Flavonol 3-O-D-galactoside	Flavonol 3-O-D-glucoside	Mitoflaxone	Rosinidin	Pulchellidin	FLAVONE	7-Hydroxyflavone	Chrysin	Quercetin	Anthocyanin 3'-O-beta-D-glucoside	Flavylium
Mass	241.2	296.1	238.2	400.3	400.3	280.2	315.2	317.2	222.2	238.2	254.2	302.2	385.3	207.2
logP	3.818	4.437	3.165	0.638	0.638	3.087	3.514	2.917	3.460	3.165	2.871	1.988	1.559	4.380
H-bond acceptors	3	3	3	8	8	4	6	7	2	3	4	7	7	1
H-bond donors	2	2	1	4	4	1	3	5	0	1	2	5	4	0
Rotatable bonds	3	4	1	4	4	3	3	2	1	1	1	1	4	1
PSA	49.33	49.33	50.44	129.59	129.59	67.51	92.29	123.52	30.21	50.44	70.67	131.36	112.52	13.14
RO5 violations	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RO3 violations	1	2	1	5	5	3	4	4	1	1	2	4	5	1
Cox2 docking score	**-8.5**	**-8.4**	**-8.7**	**-7.3**	**-8.1**	**-7.6**	**-7.3**	**-7.6**	**-9.4**	**-9.3**	**-8.9**	**-7.9**	**-7.4**	**-9.0**
Cox1 docking score	**-8.4**	**-7.8**	**-8.5**	**-6.9**	**-7.6**	**-8.1**	**-6.5**	**-5.2**	**-8.7**	**-8.6**	**-8.0**	**-6.7**	**-6.4**	**-9.1**
Optimum range score	8	8	8	8	8	8	9	9	8	8	8	8	8	8

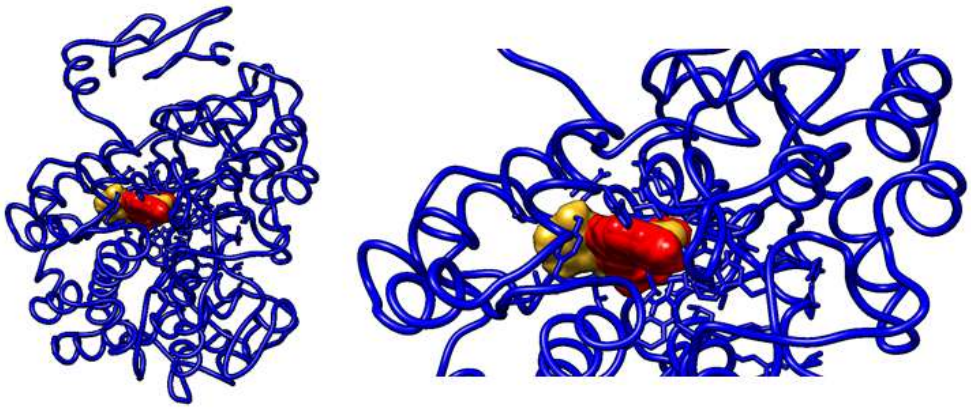


Fig-3: Cyclooxygenase-2 (Cox-2) enzyme (PDB ID: 5kir) active site pose (blue) in complex with both Rosinidin (red) and Pulchellidin (gold). Each inhibitor has its own attachment orientation.

DISCUSSION

It is known that the traditional non-steroidal anti-inflammatory drugs mode of action is by inhibiting both cyclooxygenases isoforms COX-1 and COX-2 enzymes, so finally it will block the synthesis of prostaglandins [13]. On the other hand, the gastrointestinal adverse effect of non-steroidal anti-inflammatory drugs is majorly due to the decrease in synthesis of the gastroprotective prostaglandins ($PGI_{2)}$ and ($PGE_{2)}$, which is mainly produced by COX-1 [14].

In addition, it is considered that prostaglandins are important regulators of vascular tone, water, and salt balance, and renin release so that the weakly selective non-steroidal anti-inflammatory drugs have adverse effects, those including salt retention and reduced GFR, which may elevate BP or make pre-existing hypertension worse [15].

These data concluded the need to study cyclooxygenase isoforms differences and find agents selected COX-2 strongly and not able to inactivate COX-1, this definitely will aid in inflammation management with less harmful effects on humans. The current study focused on cyclooxygenase differences and its impact on flavonoids feature as natural selective inhibitors.

The three-dimensional structure superposition of the tow cyclooxygenase isoforms 1 and 2 molecules reveals the similarity between them, with root mean square deviation RMSD = 0.892, as shown in figure 1. Although the active site of these enzymes falls in the same locations with similar structure and amino acids compositions, as shown in figure 2, a higher selectivity of ligands toward COX-2 still needed to gain the best action in inflammation suppression combined with fewer side effects. This happened because of each enzyme work mainly in different locations in the body. The COX-1 is expressed in most tissues, whereas COX-2 usually is absent, but is induced by numerous physiologic stimuli [16].

As shown in figure 2, substitution of some amino acids within active site of both cyclooxygenase molecules, in COX-2 than COX-1 Leu503 to Phy503, Val434 to Ile,Val523 to Ile, Arg513 to His513 respectively, while the other components of the active site (Arg120, Ser350, Trp387, Tyr385, , Leu384, ,Glu524) still unchanged. These substitutions are the major point to be considered in designing novel COX-2 selective inhibitors.

On the other hand, the current study investigated the probability of selected flavonoids to act as COX-2 inhibitors and its selectivity. Through tables 1, 2 and 3 which demonstrate some flavonols, flavones, and anthocyanin as a natural compound found in several plants in addition to those used in feeding, as potential inhibitors, some of them showed a high affinity to COX-2 active site attachment (summarized in table 4), while the others were not.

Within those poses strong affinity to COX-2, three of them has relatively low affinity to COX-1, the following anthocyanins flavonoids, the first one is rosinidin and pulchellidin, with COX-2 docking score -7.3 , -7.6 respectively, and COX-1 docking score -6.5, -5.2 respectively, additionally these compounds has no violations to Lipinski's role of five. These data led to suggest some flavonoids to act as selective inhibitors and these data serves the wet lab with needed data to complete researchers way led drugs discovery.

Additionally, clinical trials for humans studied the impact of flavonoids on inflammation markers are imperfect and focused on flavonoid-rich foods, not on pure flavonoids compounds. And most of those studies lack estimation of flavonoid levels, absorption and not correlate the inflammation status with levels of flavonoids in blood. So that there is a strong need for clinical trials based on pure flavonoid compounds to spot light on such flavonoids compounds role combating inflammation process [17].

Furthermore, several kinds of researches suggested that flavonoids action via numerous pathways weaken inflammation and act as probable cardioprotective, and neuroprotective [18].

In conclusion, according to current study result, some flavonoids may have the inhibitory action against cyclooxygenase isoforms; most of them may have not sufficient selectivity toward cyclooxygenase-1 making it safer for systemic usage as COX-2 inhibitors. Further investigations needed to enhance the properties to be used as optimum therapeutic agents.

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