EXCELLENT PUBLISHERS

Insight into Quercetin for it's Anti-Toxic Activity from Molecular Docking Studies

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KEYWORDS	A B S T R A C T
Quercetin, Anti-toxicity, Molecular Docking, Erythrocytes, Binding Energy, Antioxidant	Quercetin is an important flavonoid of plant origin that has various health benefits due to its anti-toxic and antioxidant role. Our previous *in vitro* wet lab studies demonstrated its protective role against petrol exhaust nanoparticles induced oxidativestress, lipid peroxidation and inflammation in rat erythrocytes. The current research explores the possible reasons and mechanisms for its anti-toxic activity through the procedure of molecular docking**.** Molecular docking was performed using the molecular modeling software "auto dock". The studies were done to evaluate the binding affinity characteristics of quercetin with antioxidant markers such as Glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT), Glutathione S transferase (GST), lipid peroxidation marker such as malondialdehyde content (MDA) and pro-inflammatory markers like Tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6),in an attempt to understand the mechanism of action of quercetin. The docking energy and hydrogen bonds were tabulated. Dockingscores indicated the application of quercetin as a potential, natural therapeutic agent to combat oxidative stress, inflammation and lipid peroxidation in rat erythrocytes.

Introduction

Flavonoids are classes of phenolic compounds that widely occur in plants. The daily average intake of flavonoids worldwide is 23mg of which, flavonoid quercetin contributes to 16mg (Hertog *et al*., 1993). Quercetin 4'glucoside and quercetin-3-rutinoside are two important classes of quercetin available extensively in plant based foods (Engelhardt *et al*., 1992).

Quercetin acts as an antioxidant by chelating metal ions and by scavenging free radicals (Morel *et al*., 1993; Leopoldini *et al*., 2006).

The red blood cells (RBC's) are the main transporters of oxygen to all tissues from the lungs. In the systemic circulation, the RBC's are continuously vulnerable to attack by free radicals, such as reactive oxygen species

(ROS), which are formed as a result of exhaustive exercise, disruption in normal cellular events or on exposure to external environmental agents. This causes impairment of RBC function and leads to RBC oxidative stress that further induces red blood cell ageing (Nagababu *et al*., 2003). Under normal conditions, the RBC's are well equipped with a potential antioxidant defense machine of both enzymatic and non-enzymatic origin (Stern, 1985).

However, they show many structural disruptions and alterations of the antioxidant system following exposure to environmental toxins and chemicals. This was evident from our previous wet lab studies on rat RBC's. On exposure to environmental air pollutant toxins such as the diesel and petrol exhaust nanoparticles, the rat RBC's demonstrated a significant decrease in antioxidant enzyme levels (SOD, CAT, GSH and GST) and a significant increase in malondialdehyde (MDA) levels, thus contributing to oxidative stress and lipid peroxidation in RBC's. Prior treatment with quercetin showed replenishment of antioxidant level and brought the values to near normal, hence proving the protective effect of quercetin.

This clearly demonstrated that these environmental nanoparticles lead to RBC oxidative stress with the plasma membrane as the preliminary site of damage and hence the peroxidation of lipids on the plasma membrane caused cross linking of protein and lipid molecules to various extends along with hemolysis (Durga *et al.,* 2015), thus contributing to oxidative stress and lipid peroxidation in rat RBC's.

In this context, *in silico* investigations continue to be a great promise for evaluating the interaction energies between a known compound and a target ligand. In view of the above, the present study merits in exploring the potential role of quercetin as an anti-

toxic and anti-inflammatory agent based on the binding pattern, hydrogen bond formation and energy values.

Materials and Methods

In this research, "auto dock" software was used for molecular docking studies.

Protein structure preparation

Structures of proteins such as SOD, CAT, GSH, GST, MDA, TNF-α and IL-6 were retrieved from the RCSB protein data bank (http://www.rcsb.org/pdb/home/home.do). The proteins were prepared by the addition of hydrogen atoms, identifying overlaps, assigning bond order and by deleting unwanted chains, cofactors and water molecules. Following this the grid generation was done.

Ligand structure preparation

The quercetin ligand used in the present study was obtained from previous literature (Watson *et al*., 2000). Chem Sketch, (chemically intelligent drawing interface freeware), developed by Advanced chemistry development, Inc, was used in ligand structure construction.

Using Chem sketch draw mode, the ligand was constructed, 3D optimizations were performed and then the ligand was saved as mol file. Following this, TORSDOF was used to calculate the free energy change upon binding. After all conditions were set, the above ligand was saved in "pdbq" format.

Macromolecule preparation

For **"**Autodock", the file format was converted and saved as "pdbqt" format, following grid parameter file preparation.

Docking studies

After the grid generation, the processed ligand was docked with the protein to evaluate the interaction between each target protein and the ligand. Interactions were hydrophilic, Vander Waals and hydrophobic. The interaction strength varies from protein to protein based on its affinity for the ligand. During the auto-docking procedure, the ligand conformation was retained, followed by the extra precision mode selection.

Visualization and analyzing docking results

Once the target ligand was docked against all proteins of interest, the results were visualized for their interactions, binding energy, H-bond formation and few other parameters using the Accelrys discovery studio visualize 2.5.

Results and Discussion

Flavonoids are plant phenolics that have various beneficial effects on mammalian structures and cellular systems (Melzig, 1996) and have proved be protect our biological membranes and cells against oxidative damage induced by free radicals (Kitagawa *et al.*, 1992).

The RBC's contain lipids that are rich in unsaturated fatty acids. They are more prone to oxygen exposure than other body cells and tissues and are thus more frequently exposed to oxidative damage. The RBC membrane is also easily invaded by peroxidants, that lead to cell hemolysis. Moreover, the protein hemoglobin present in these erythrocytes is a very strong catalyst which initiates lipid peroxidation in RBC's. Along with these parameters, the pro-inflammatory cytokines were also found to be elevated on exposure to environmental toxins in the RBC's leading to inflammation (Durga *et al.*, 2015).

The antioxidants protect the RBC membranes from oxidative damage due to environmental toxins (Sarkar *et al.*, 1997) and also they are capable of preventing oxidative damage that is caused as a result of the RBC's inability to scavenge ROS such as superoxide anions and hydroxyl radicals (Galati *et al.*, 2002). Studies in humans and animals suggest that dietary flavonoids can reduce the risk of cancers (Arta *et al.*, 2001), cardiovascular diseases (Liu *et al.*, 2000; Hertog *et al.*, 1993; Sesso *et al.*, 2003) and cerebrovascular diseases (Knekt *et al.*, 2002). Quercetin is a flavonoid found widely in black tea, green tea and other plant based foods and it has been reported to have diverse physiological effects due to their anti-oxidative ability (Kalender *et al.*, 2012, Baba *et al.*, 2001). Our previous wet lab studies proved quercetin, as a potent antioxidant that directly scavenges free radicals, alters antioxidant pathway *in vitro* and inhibits LPO in RBC's. Quercetin was also able to protect the erythrocytes from cellular senescence and oxidative damage in a dose dependent manner (Durga *et al.*, 2015).

The current research aims to understand the mechanism of action of chosen flavonoid (quercetin). Molecular docking studies were performed by *in silico* methods, using "Auto dock" software. Initially the 3D ligand structure was generated using ACD Chem sketch and (Figure 1a & 1b) followed by docking studies. The ligand-protein docking studies aim to determine the dominant binding models of a ligand with the protein of known 3D structures (Mittal *et al.*, 2009). The current docking study yielded important information about the orientation of the ligand in the various binding pockets of the target proteins. Several potential drugs have

been evaluated and identified by the method of docking simulation.

The structure-function relationship of quercetin was analyzed to understand its biological activity against the target proteins such as SOD, CAT, GST, GSH, MDA,

TNF-α and IL-6 using the 3D structure of the proteins obtained from the PDB site of these proteins (Figure 2 & Figure 3). Details of the proteins chosen for the study is given in Table 1. The ligand details are tabulated in Table 2.

Table.1 Details of the proteins

S.NO	PROTEIN	PDB ID	CHAIN	RESOLUTION
1	MDA	3B6E	A	1.6 Å
2	GSH	5hv9	A	3.0 Å
3	IL-6	4ZS7	A	2.93 Å
4	TNF-α	4TWT	B	2.85 Å
5	GST	5A1N	A	2.1 Å
6	SOD	2XJK	A	1.45 Å
7	CAT	5DLZ	A	1.7 Å

Table.2 Quercetin Lipinski's rule

Name of the compound	Alternative name	Molecular weight	Molecular formula	H-Bond donor	H-Bond acceptor	Xlogp3 value (& it ;=5)	Description
Quercetin	Quercetol, Quercitin, Quercetin, Sophoretin, Quertine.	302.2357 g/mol	$C_{15}H_{10}O_7$	5	7	1.68	It's a heterocyclic phenolic flavonol widely present in plants.

Table.3 Docking scores and number of hydrogen bonds formed between the proteins and quercetin compound

S.No	Proteins	Key Residues	Docking Energy (Kcal/Mol)	H-Bond
1	SOD	Pro^{28}, Glu^{21}, Lys^{23}	-4.88	1
2	CAT	Glu^{49}, Arg^{113}, Ter^{113}	-5.45	2
3	GSH	Arg^{96}, Gln^{102}, Ala^{106}	-4.54	2
4	GST	Glu^{34}	-3.29	1
5	MDA	Lys^{350}, Gln^{355}, Gly^{357}	-7.66	1
6	TNF-α	Glu^{116}, Typ^{116}, Pro^{100}, Gln^{102}, Arg^{103}, Cys^{69}	-5.43	5
7	IL-6	Leu^{133}, Gln^{127}, Asn^{132}, Ala^{130}	-6.52	3

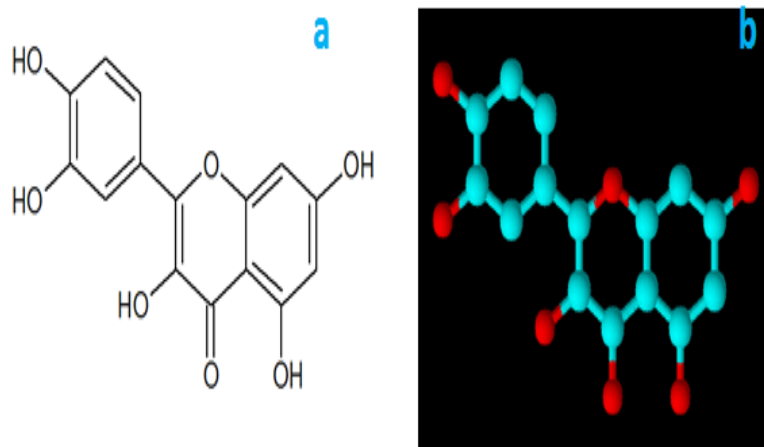**Fig.1** (a & b): 2D and 3D structures of ligand molecule (Quercetin)

Fig.2 Structure of target protein molecules (Antioxidants). (a)Represents SOD,(b) represents CAT, (c) represents GSH and (d) represents GST.
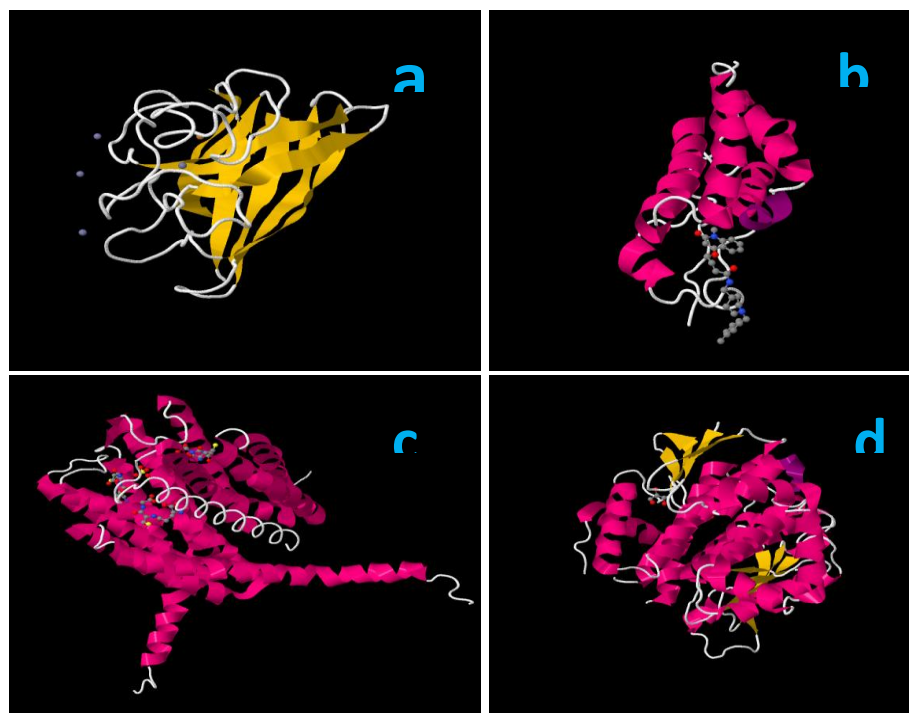(Thepink color represents -helix, yellow color -beta sheets&the white color represents - turns)

Fig.3 Structure of target protein molecules. (a) represents MDA, (b) represents TNF- α and (c) represents IL-6(The pink color represents - helix, yellow color - beta sheets & the white color represents - turns)
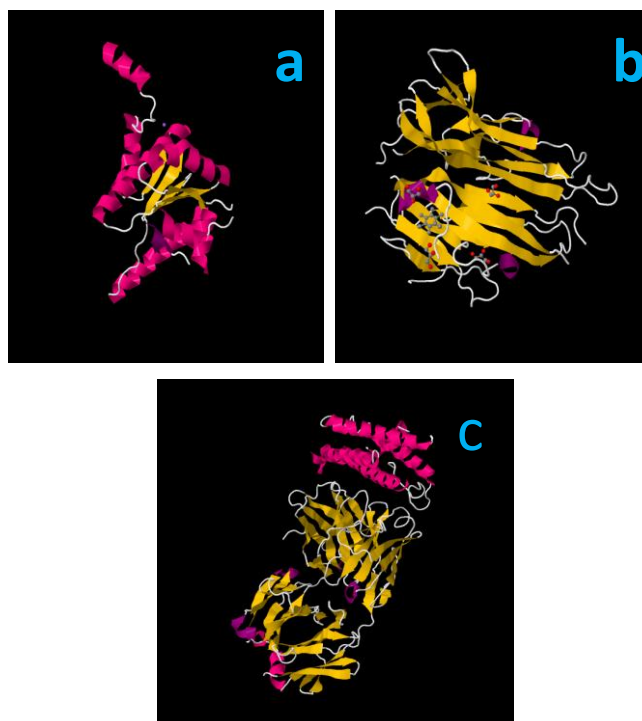

Fig.4 (a & b): Binding pattern of ligand molecule (quercetin – blue color) with target antioxidant proteins (Pink color). (a) represents 'SOD' and (b) represents 'CAT'.
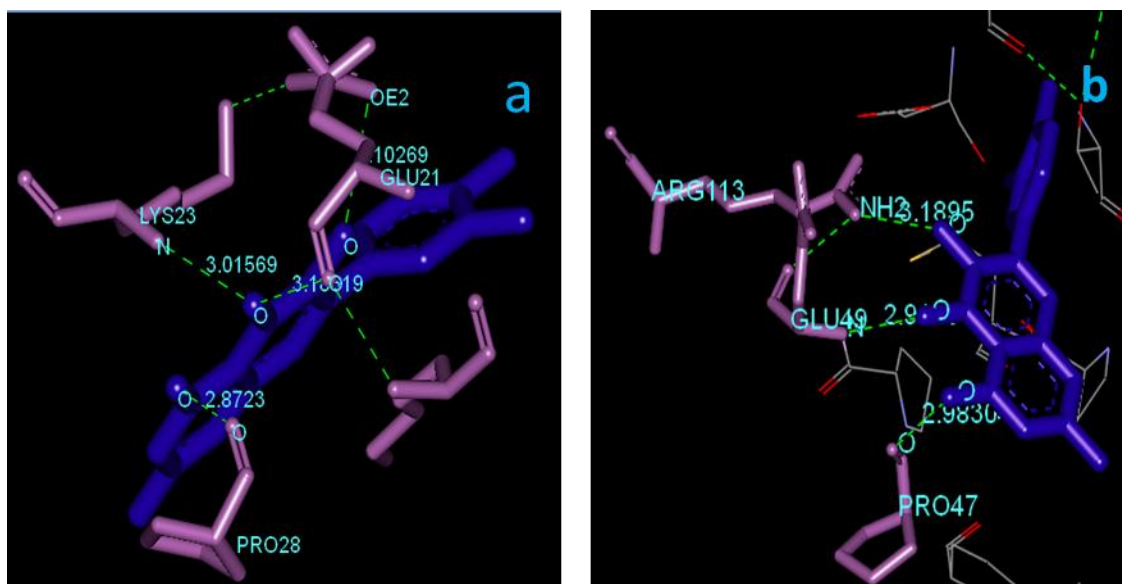
Fig.5 (a & b):Binding pattern of ligand molecule (quercetin – blue color) with target antioxidant proteins (Pink color). (a) represents 'GSH' and (b) represents 'GST'
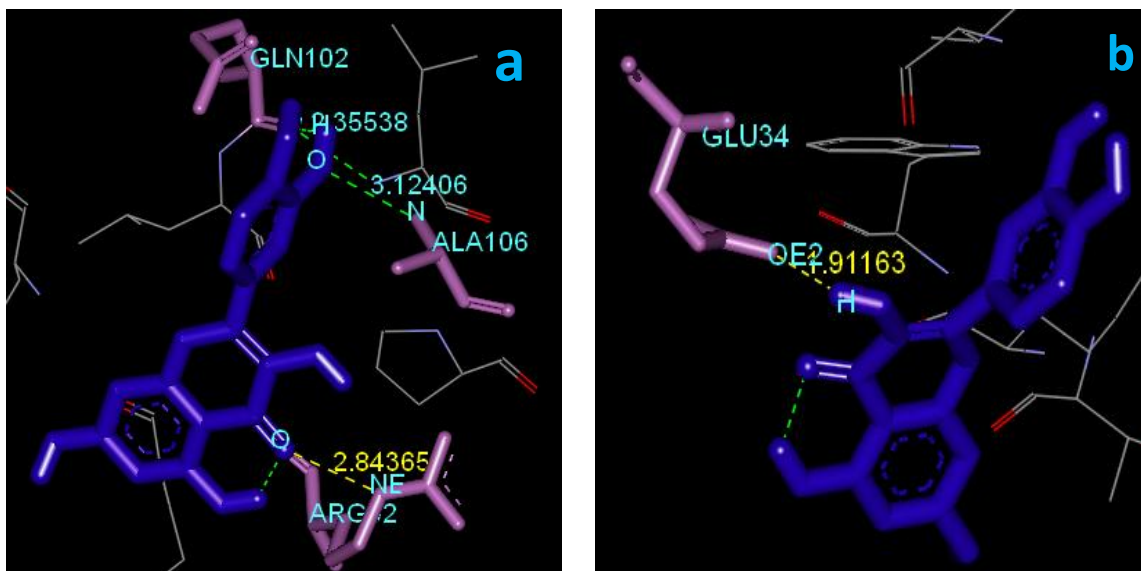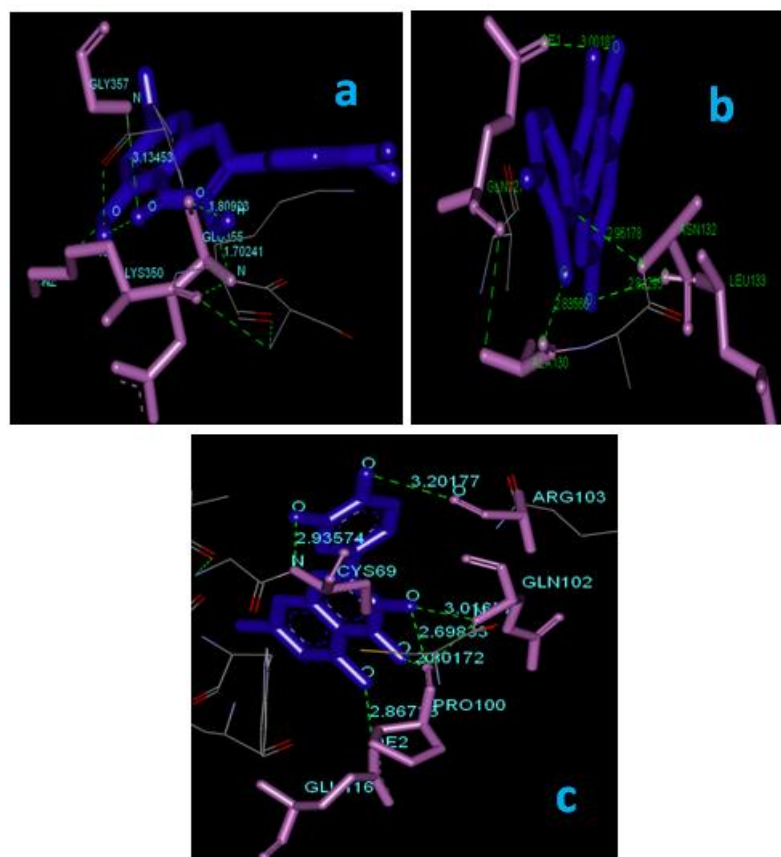

Fig.6 (a, b & c): Binding pattern of ligand molecule (quercetin – blue color) with the proteins (Pink color). (a) represents 'MDA', (b) represents 'IL-6' and (c) represents 'TNF-α'.

Evaluation, of the pattern of binding between the target protein and ligand suggested that the binding pattern varied with the protein nature. This was explored based upon our observation that quercetin interacted with antioxidant enzyme SOD with its amino acids Pro^{28}, Glu^{21} and Lys^{23}(Figure 4a) while with CAT amino acids at Glu^{49}, Pro^{47} and Arg^{113} (Figure 4b),with GSH amino acid at Arg^{96}, Ala^{102}, Gln^{106} (Figure 5a) and with GST amino acid at Glu^{34} (Figure 5b) alone. In contrast to this with MDA, quercetin was found to bind at Lys^{350}, Glu^{355}, Gly^{357} amino acid alone (Figure 6b) and with the anti-inflammatory protein TNF-α binding of quercetin was visible at amino acid residueGlu^{116}, Arg^{103}, Cys^{69}, $Gln^{102}$and Pro^{100} (Figure6a), whereas for IL-6 binding was observed at Gln^{127},Asn^{132},Leu^{133} and Ala^{130}, respectively (Figure6c).

These docking data's revealed that ligand quercetin interacts with the target proteins at different amino acid residues and mechanism of binding pattern varies from protein to protein. The critical assessment of the binding nature of quercetin further demonstrated that the number of hydrogen bonds formed between the ligand and target protein also varied extensively (Table 3). Antioxidant enzymes such as SOD, CAT, GSH and GST formed 1, 2, 2 and 1H-bond respectively. In contrast to this, the lipid peroxidation marker MDA, formed 1 bond and the anti-inflammatory cytokines formed 5 (TNF-α) and 3(IL-6) bonds, respectively with the ligand quercetin.

The antioxidant and anti-inflammatory activity of quercetin observed in association with SOD, CAT, GSH, GST, MDA, TNF-α and IL-6 proteins further demonstrated that the chosen compound could be potentially used as an anti-toxic agent. This comparative data analysis of literature and the current investigation further demonstrated that the compound influences the conformation of the target proteins by interaction at various places other than the active site and thus induces anti-toxic action (Krishna *et al*., 2013).

In accordance to the above data, the docking scores were measured and viewed considering the electrostatic and steric properties. The binding pattern of target proteins with the ligand quercetin was expressed as kcal/mol (Muthukala *et al*., 2015). The data showed that the binding pattern differs with the protein and thus also influences the docking score value. Higher fitness score of -3.29,-4.54 and -4.88 were observed for antioxidant enzymes GST, GSH and SOD. In contrast to this fitness score of -5.45 was observed for CAT enzyme. Whereas for TNF-α, IL-6 and MDA fitness scores of -5.43, -6.52 and -7.66 were observed. Thus, based upon docking studies, quercetin can be used as a potential anti-toxic agent against toxicity induced in the erythrocytes. This current study, thus acts as a supportive evidence for our previous wet lab studies.

In conclusion, novel drugs or compounds with potential biological activity is a quick need today. In the current study, different target proteins were successfully docked with the ligand (quercetin) to study the mechanism of drug interaction and to track the current race between drug and their development, especially for new compounds using molecular methods. The docking score of quercetin was calculated using "auto-dock" software. Though the pattern of binding between the ligand and each protein varied, docking scores indicate that quercetin has potential anti-toxic activity. Thus, we conclude that regular dietary intake of quercetin can act as a significant anti-toxic molecule against toxicity induced in erythrocytes.

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