

Noncompetitive Inhibition of Bovine Liver Catalase by Lawsone: Kinetics, Binding Mechanism and *in silico* Modeling Approaches

Simin khataee^a, Gholamreza Dehghan^a, Samaneh Rashtbari^a, Siavash Dastmalchi^{b,c} and Mehrdad Iranshahi^d

^aDepartment of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

^bBiotechnology Research Center, School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. ^cFaculty of Pharmacy, Near East University, POBOX: 99138, Nicosia, North Cyprus, Mersin 10, Turkey. ^dBiotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

Lawsone (2-hydroxy-1,4-naphthoquinone; LAW), as a naphthoquinone derivative, is the biologically active component of *Henna* leaves. In this study, the structural and functional effects of LAW on bovine liver catalase (BLC), has been studied utilizing ultraviolet-visible (UV-vis) absorption, fluorescence, and ATR-FTIR spectroscopic techniques, and molecular docking approach. *In-vitro* kinetic study showed that by adding gradual concentrations of LAW, catalase activity was significantly decreased through noncompetitive inhibition mechanism. UV-vis and ATR-FTIR spectroscopic results illustrated that additional concentration of LAW lead to significant change in secondary structure of the enzyme. The fluorescence spectroscopic results at different temperatures indicated that LAW quenches the intrinsic fluorescence of BLC by dynamic mechanism and there is just one binding site for LAW on BCL. Changing the micro-environment nearby two aromatic residues (tryptophan (Trp) and tyrosine (Tyr)) were resulted from synchronous fluorescence. The thermodynamic parameters were implied that the hydrophobic bindings have a significant impress in the organization of the LAW-catalase complex. Molecular docking data in agreement with experimental results, confirmed that hydrophobic interactions are dominant. Inhibition of enzyme activity by LAW, showed that along with its helpful effects as an anti-oxidant compounds, the side effects of LAW should not be overlooked.

Keywords: Bovine liver catalase; Lawsone; Molecular docking; Naphthoquinones; Noncompetitive inhibition.

Introduction

Catalase (H₂O₂ oxidoreductase, EC 1.11.1.6; CAT)(PDB ID 1TGU), is an antioxidative and protective enzyme that plays critical role in organelles and tissues to detoxify the impacts of hydrogen peroxide (H₂O₂)(1, 2). It is a ubiquitous enzyme of almost all aerobic organisms and many anaerobic organ-

isms (3). Catalase deficiency leads to the steady state concentration of H₂O₂, which may raise various pathological conditions as cardiovascular disease, cancer, ageing oxidative, hypertension, Alzheimer, diabetes mellitus, and acatalasemia (4, 5).

Catalase from Bovine liver (BLC) (MW = 240000 Da) is a homotetramericheme-containing enzyme, with four equal-size subunits. Each subunit (MW = 60 kDa) of this enzyme organized of 253 pairs of amino acids,

* Corresponding author:

E-mail: dehghan2001d@yahoo.com

one NADPH as a cofactor (per subunit) and heme prosthetic that are strongly connected to the structure of CAT through tyrosine, histidine, and asparagine (Y415, H75 and N201) as ligand(6).

Also each subunit divides into four domains: a conserved eight-stranded β -barrel which generate the hydrophobic core per subunit, wrapping loop which attaches the β -barrel to N-terminal threading arm, C-terminal helices which surround the β -barrel, and N-terminal threading arm(7). Catalase structure has central cavity and three access channels that convert substrate to the active site. The channels provide substrate access to active site and allow release of product(8).

BLC, can strongly bind to NADPH (as cofactor (in the middle of the β -barrel and the helical domain (9). The function of the NADPH is reducing the susceptibility of enzyme to prevent inactivation of CAT at low concentrations of its substrate (10). NADPH plays the role of an electron donor by transformation of compound II to restore the active form of the catalase (11). In accordance with BLAST (Basic Local Alignment Search Tool) consequences, bovine liver catalase includes efficient similarities with human catalase (12). So, BLC can be utilized as a model enzyme of human liver CAT.

Naphthoquinones are ubiquitous in nature and they have been seem to have a lot of biological activities and medicinal properties, such as cytotoxic, anti-oxidant, anti-cancer, anti-fungal, anti-bacterial, and anti-tumoral effects,(13) which can play a main role in the human defense system (14). 1,4-Naphthoquinones, as anti-cancer compound are found in a number of aminoquinones such as actinomycins, streptonigrin, etc (14, 15). An important derivative of 1,4-naphthoquinone is lawsone (LAW). LAW (2-hydroxy-1,4-naphthoquinone), the active component of Henna leaves (*Lawsonia inermis* Linn), has a various kind of biological activities such as antioxidant, antibacterial, antifungal, anti-inflammatory, antipyretic, and anticancer. It is a red-orange pigment and partially water-soluble (14, 16). The chemical formula of LAW is $C_{10}H_6O_3$ corresponding to the total molecular weight of 174.16 atomic mass units. In the chemical

structure of LAW, two oxygen atoms are connected to the carbons of naphthalenic at positions of 1 and 4 for generation of 1, 4-naphthoquinone. A hydroxyl group ($-OH$) of enolic temper is present at the position 2 (Figure 1)(16, 17).

Reactive oxygen species (ROS), extremely reactive molecules, exist in elevated levels in cancer cells because of their high metabolic activity and play a main role in carcinogenesis through induction of DNA damage. Cells detoxify ROSs and maintain intracellular homeostasis by using different antioxidant enzymes including catalase, glutathione peroxidase and superoxide dismutase (SOD) (18). Decreased antioxidant enzymes activity can be associated with different types of cancer (19). Literatures support anticancer potential of naphthoquinone derivatives such as LAW in various types of cancer. However, it has been reported that the increase in ROS can induce tumor cell death through damaging function of ROS and induction of apoptosis in tumor cells through death signaling pathways. So, studying effects of different compounds on the activity of antioxidant enzymes and induction of oxidative stress by increasing ROS, can be one of the main and interesting issues in cancer therapy. In the present study the impact of LAW on the function and structure of CAT, as a most important antioxidant enzyme, was studied *in-vitro* using multiple spectroscopic methods and molecular docking technique and its therapeutic potential was investigated in treatment of cancer by decreasing CAT activity.

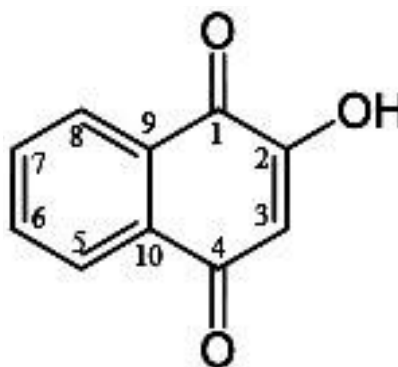


Figure 1. Chemical structure of lawsone (2-hydroxy-1,4-naphthoquinone).

Experimental

Materials

Catalase from bovine liver and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Company (St Louis, Missouri). LAW was obtained from Prof. Iranshahi's laboratory, Mashad University of Medical Science. Sodium phosphatesalts and hydrogen peroxide (H_2O_2) 30% were acquired from Merck Co (Darmstadt, Germany). The LAW stock solution was freshly prepared by dissolving it in DMSO and was mixed with sufficient phosphate buffer to obtain the desired volume. The concentration of H_2O_2 stock solution was accounted spectrophotometrically through the molar extinction coefficient of $40\text{ M}^{-1}\text{cm}^{-1}$.

Methods

Kinetics studies

Catalase (4 nM) activity was assessed by following the decline in the maximum absorbance of H_2O_2 at 240 nm and at 25 °C, based on its decomposition by the enzyme using a UV spectrophotometer (T-60, PG Instrument UK)(3). The assays were accomplished in 3 mL cuvette, including 50 mM sodium phosphate buffer (pH 7), different concentrations of H_2O_2 (10-70 mM) and 10 μL of CAT suspension (11). In order to study the structural and functional alterations of enzyme by LAW, CAT into the reaction medium was incubated with different concentrations of LAW (1.42, 2.84, 5.68, 11.36 and 17.04 μM) for 3 minutes. Subsequently, an appropriated concentration of substrate (55.5 mM) was added to the mixture and the alteration in the absorbance was monitored in A_{240} .

Fluorescence measurements

In order to assess the three-dimensional conformational changes of CAT through increasing concentrations of LAW (0.47, 4.7, 11.8, 14.2, 23.6 and 37.8 μM), fluorescence intensity evaluations were recorded utilizing a FP-750 Jasco spectrofluorometer (Kyoto, Japan) with 1 cm quartz cells(20). The excitation wavelengths (λ_{ex}) fitted at 280 nm and the fluorescence spectra were recorded from 300 to 500 nm (λ_{em}) at two temperatures (25, 30 and 37 °C).

Synchronous fluorescence spectra of BLC

were evaluated using $\Delta\lambda=15\text{ nm}$ ($\lambda_{\text{em}}=270-330$) and $\Delta\lambda=60\text{ nm}$ ($\lambda_{\text{em}}=310-380$) to obtain the fluorescence spectra of tyrosine (Tyr) and tryptophan (Trp) residues, respectively. The slit widths and the scan speed of both emission and excitation were set at 10 nm and 1000 nm min^{-1} , respectively.

UV-vis and ATR-FTIR spectroscopies

The UV-vis spectrophotometer (T-60, PG Instrument UK) was utilized to assess the UV-vis absorption spectra of CAT in the spectral range of 200-500 nm, without and with gradual concentrations of LAW.

To elucidate the protein-ligand binding feature, Fourier transform infrared spectroscopy (FT-IR) was carried out with a Bruker, Tensor 27 FT-IR spectrometer equipped with a KBr and ZnSe (beam splitter), Attenuated Total Reflection (ATR) technique with resolution of 1 cm^{-1} and DLa TGA detector. The FT-IR spectrum of native CAT was obtained in sodium phosphate buffer, at 1200 cm^{-1} to 1800 cm^{-1} and compared with the results of CAT-LAW complex spectra (9.4 and 23.6 μM of LAW).

Molecular docking investigation

The molecular docking analysis was done to detect the best binding site of CAT for LAW using AutoDock 4.2 software. The Protein Data Bank (RCSB) was used to take crystallography structure of CAT. The 3D structure of LAW was drawn by Hyperchem 8.0.6 and the minimizing of the energy was performed by Gaussian 98 program (21-23). The Lamarckian genetics algorithm technique was used to search connection site of LAW on CAT. Finally, The Auto Dock Tools 1.5.4 package were used to provide the analyzed mode of binding between LAW and CAT (3).

Results and Discussion

Functional effects of LAW on CAT (kinetics studies)

To study the impact of LAW on the function of CAT, the UV-vis spectrophotometer was applied. At first, the kinetic parameters of CAT were determined by assaying enzyme in the presence of incremental amounts of H_2O_2 (10-70 mM) and appropriate concentration of

CAT (4 nM). The rate of decomposition of substrate by CAT was evaluated at 240 nm and reduction in H₂O₂ absorption per unit time that was considered as enzyme function at 25 °C. Based on our previous studies, BLC activity was increased with raising the substrate concentration from 10 to 55.5 mM. Addition of higher concentration of H₂O₂ (more than 55.5 mM) significantly reduced enzyme activity due to inhibitory effect of substrate(21). Different kinetic parameters of CAT have been calculated by Lineweaver-Burk plot, as it is shown in Figure 2 and equation 1.

$$\frac{1}{V_{\max}} = \frac{k_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}} \quad (1)$$

The values of K_m , Michaelis-Menten constant, and V_{\max} for the free enzyme were obtained as 35.92 mM and 2.39 mM.S⁻¹, respectively. Also, the value of K_{cat} , catalytic constant was determined as 5.9×10^5 s⁻¹, using equation (2):

$$V_{\max} = K_{cat} [E_t] \quad (2)$$

where, [Et] incorporates the total CAT concentration.

To identify the alterations in the function of CAT by LAW, the enzyme activity was evaluated in the presence of increasing

concentrations of LAW (1.42, 2.84, 5.68, 11.36 and 17.04 μM) and optimum concentration of H₂O₂ (55.5 μM). The results of this experiment showed that an increase in the concentrations of LAW cause a significant decrease in the enzyme activity. The value of IC₅₀, was approximated 9.3 μM using Figure 3A.

Three different types of reversible inhibitory mechanism for the enzyme have been defined: (a); competitive inhibition in which the inhibitor and free enzyme connect to each other through enzyme active site (b); noncompetitive inhibition in which inhibitor connects to both the free enzyme at a site that is different from the substrate binding site and the enzyme-substrate complex and (c); uncompetitive inhibition in which inhibitor can bind reversibly to the enzyme-substrate complex to yield anticompertitive inhibition (24). To realize the inhibitory mechanism of CAT by LAW, alterations in the enzyme activity were measured after forming the complex with different concentrations of LAW (4.6, 9.3 and 18.6 μM). The Lineweaver-Burk plots were drawn (Figure 3B). The kinetic parameters (K_m and V_m) were computed using Lineweaver-Burk plots and compared with kinetic parameters of native enzyme(25). With increasing the concentration of LAW, the K_m (35.92) didn't show significant alteration in

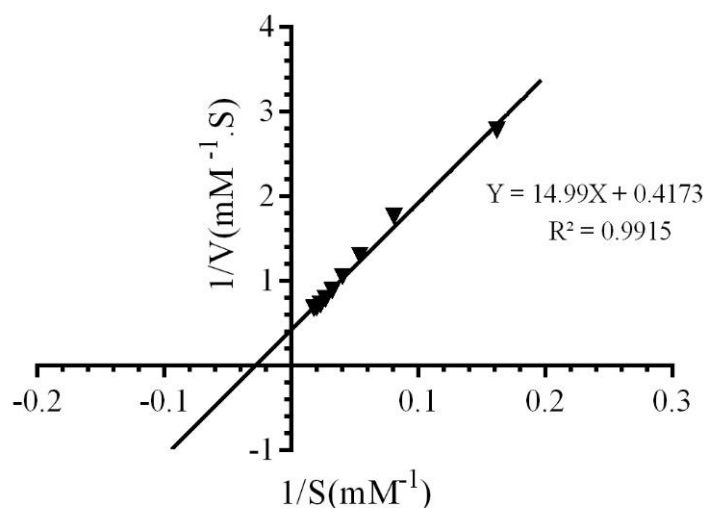


Figure 2. Lineweaver-Burk plot of bovine liver catalase activity in the presence of different concentrations of H₂O₂ (10-70mM) in 50 mM phosphate buffer, pH 7.0 at 298 K.

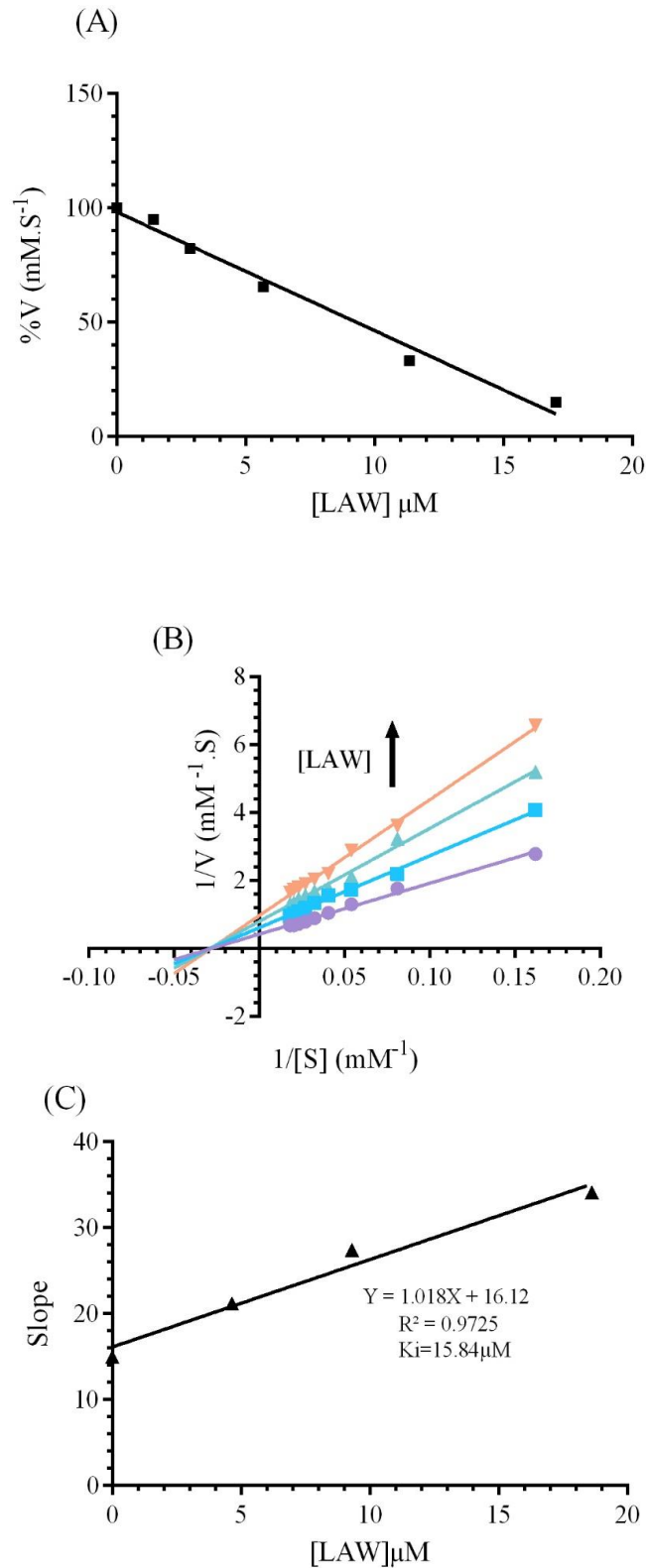


Figure 3. (A) The effect of various concentrations of LAW (0, 1.4, 2.84, 5.68, 11.36 and 17.04 μM) on the activity of BLC (4 nm) in 50 mM phosphate buffer, pH 7 at 298 k. (B) Lineweaver-Burk plot of BLC (4 nm) with and without various concentrations of LAW: 0, 4.6, 9.3, 18.6 μM in 50 mM phosphate buffer, pH 7, 298 k. (C) Secondary plot of BLC activity using the slopes of the primary Lineweaver-Burk plots versus concentrations of LAW.

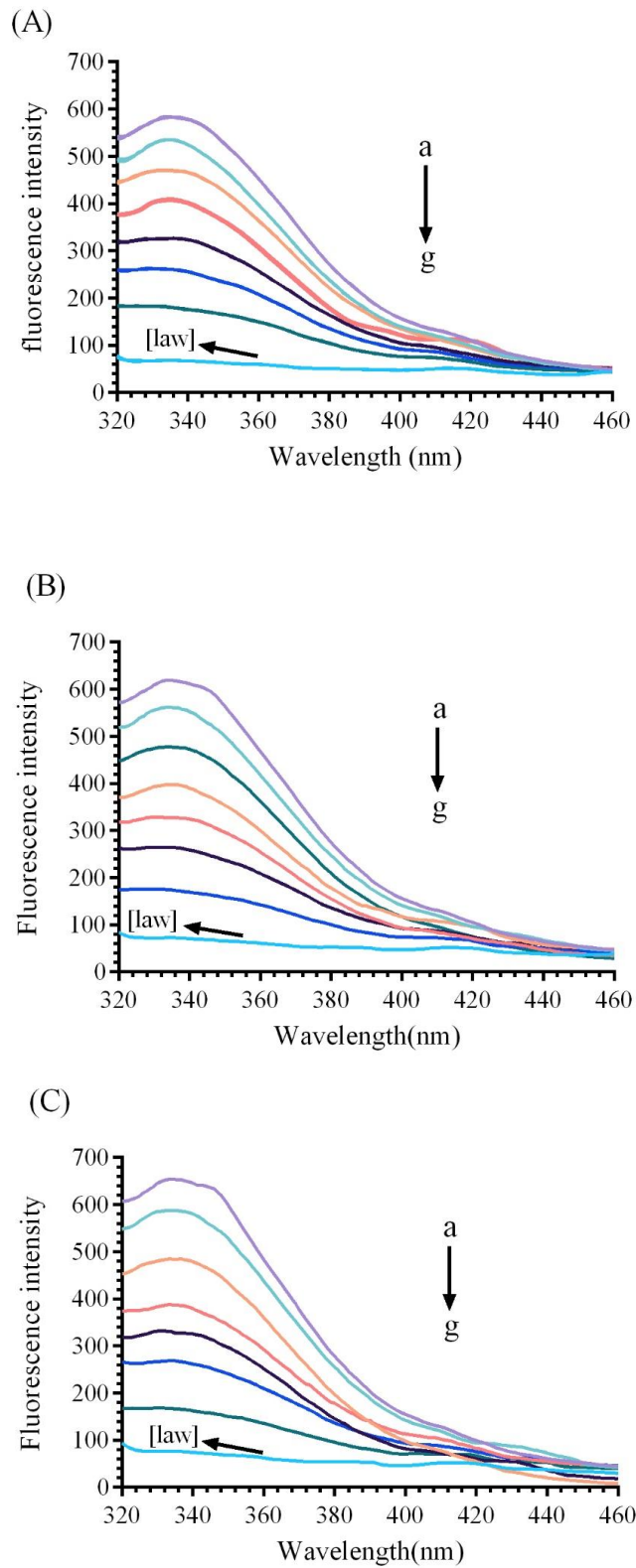


Figure 4. Fluorescence emission spectra of bovine liver catalase (BLC) in the presence or absence of different concentrations of LAW at 280 nm excitation wavelength, BLC concentration: (0.7 μ M); LAW concentrations:(a) 0, (b) 0.47, (c) 4.7, (d) 11.8, (e) 14.2, (f) 23.6 and (g) 37.8 μ M, (h) only LAW, 0.7 μ M, (A) 298 K, (B) 303 K, (C) 310 K.

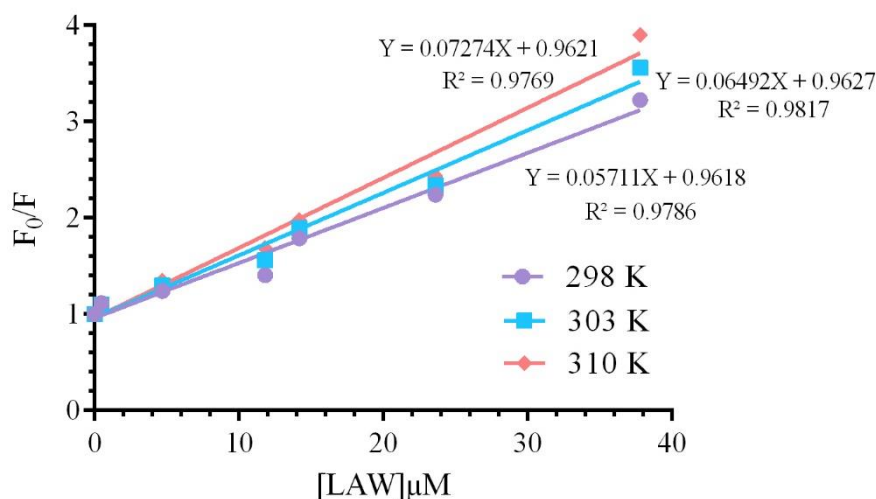


Figure 5. Stern- Volmer plots for the bovine liver catalase fluorescence quenching by LAW at 298, 303 and 310 K.

the presence of LAW, whereas values of V_{max} of the enzyme decreased from $2.39 \text{ mM}\cdot\text{s}^{-1}$ for free BLC to $1.64 \text{ mM}\cdot\text{s}^{-1}$, $1.26 \text{ mM}\cdot\text{s}^{-1}$ and $1.036 \text{ mM}\cdot\text{s}^{-1}$ for the enzyme in the presence of incremental concentration of LAW (4.6, 9.3 and $18.6 \mu\text{M}$). As a result, LAW acts as a non-competitive inhibitor for catalase. The K_i value was obtained from the equation (3) and (Figure 3c) as $15.77 \mu\text{M}$.

$$\frac{1}{V_0} = \frac{k_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \left(1 + \frac{[I]}{K_i}\right) \quad (3)$$

Fluorescence spectroscopy

The fluorescence spectroscopy was performed to predict the changes in the protein structure through interaction with various ligands. It gives valuable information around structural changes, binding mode, quenching mechanism, and binding constants (22). Fluorescence quenching is the reduction of fluorescence intensity of a fluorophore which results from some processes such as molecular rearrangements, energy transfer and etc (26). Bovine liver catalase consists of four polypeptide subunit, which each subunit contains 6 tryptophan (Trp) and twenty Tyr residues (2, 11). Three aromatic residues

(tyrosine, tryptophan, and phenyl alanine) of BCL lead to intrinsic fluorescence emission. In this experiment, the 280 nm as an excitation wavelength has been used to measure the fluorescence quenching mechanisms of CAT(21). Native enzyme shows a fluorescence emissions peak at 338 nm. As it can be observed in Figure 4, the fluorescence spectrum of the CAT gradually reduces with increasing the concentrations of LAW (0.47, 4.7, 11.8, 14.2, 23.6 and $37.8 \mu\text{M}$) at 25, 30, and 37°C .

Static and dynamic quenching, as two distinct quenching mechanisms, derive from the dependence of ligand-biomolecule interaction process on temperature. In static quenching, enhancing temperature leads to reduce the bimolecular quenching constant, but in dynamic quenching, enhancing temperature cause increase in the value of quenching constants (27). To confirm the quenching mechanism of CAT in the presence of LAW, Stern-Volmer equation (4) was used;

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (4)$$

where, F and F_0 are the fluorescence intensities of enzyme with and without the quencher (LAW), respectively, $[Q]$ is the total

Table 1. Binding and thermodynamic parameters of BLC and LAW complex at different temperatures: (25 °C, 30 °C and 37 °C).

T (K)	n	$K_{SV} (\times 10^4 M^{-1})$	$K (\times 10^3 M^{-1})$	$\Delta G \text{ kJmol}^{-1}$	$\Delta H \text{ kJmol}^{-1}$	$\Delta S \text{ JK}^{-1}\text{mol}^{-1}$
298	0.7	5.7	1.97	-18.79	10.23	98.08
303	0.7	6.4	2.2	-19.38		
310	0.7	7.2	2.6	-20.26		

concentration of the quencher (LAW), K_{SV} is the Stern–Volmer quenching constant (28). The K_{SV} value was calculated applying the F_0/F vs. $[Q]$ plot (Figure 5) at two different temperatures and are listed in Table 1. These data indicated that the values of K_{SV} increase with raising temperature, so the quenching mechanism is dynamic.

Synchronous fluorescence study

Synchronous fluorescence was applied to notify significant report about the microenvironment of CAT fluorophore groups (Trp and Tyr). In synchronous fluorescence, the spectra of molecules are taken from simultaneously scanning the excitation and emission spectra, when the wavelength intervals ($\Delta\lambda$) maintained at 15 nm and 60 nm for Tyr and Trp, respectively (29). Additional concentrations of LAW lead to gradual reduce in the fluorescence intensity of BLC indicating complex formation between BLC and LAW. As shown in Figure 6, different concentrations of LAW induce a clear red shift in $\Delta\lambda=60$ nm and very small red shift in $\Delta\lambda=15$ nm in comparison with the native CAT, implying that the Trp and Tyr are located in a polar environment.

Thermodynamic parameters and binding studies

The number of binding sites (n) and binding constant (K_a), were obtained from the equation 5;

$$\log \frac{F_0 - F}{F} = \log K + n \log [Q] \quad (5)$$

The binding constant K_a and the number of binding sites “ n ”, were calculated by using

the plot $\log \frac{F_0 - F}{F}$ vs. $\log [Q]$ (Figure 7) and the results were listed in Table 1.

K_a and “ n ” were determined by using the intercept and slope values, respectively. The number of binding sites at both temperatures (25, 30 and 37 °C) was determined approximately equal to 1 ($n \approx 1$), describing that one molecule of LAW can bind to one molecule of catalase and CAT has only one binding site for LAW. The K_a was enhanced by increasing the temperature, originated from an endothermic interaction between the CAT and LAW. The K_a value was $\approx 10^3$, illustrating that there is a strong binding force between LAW and CAT(30). There is diverse mode of interaction between proteins and ligands such as electrostatic interactions, hydrogen bonds, hydrophobic forces, and van der Waals (11). Utilizing the thermodynamic parameters including enthalpy change (ΔH), entropy change (ΔS), and free energy change (ΔG), binding forces was estimated(31): 1) $\Delta H < 0$ and $\Delta S > 0$ imply that electrostatic forces is more important, 2) $\Delta H < 0$ and $\Delta S < 0$ illustrate hydrogen bonding to play the main role in the interaction, 3) $\Delta H > 0$ and $\Delta S > 0$ suggest hydrophobic bounding is dominant and 4) $\Delta H > 0$ and $\Delta S < 0$ indicate van der Waals forces are main (21, 32). van't Hoff and the following equations (6 to 8) were used to calculate the changes in enthalpy (ΔH), Gibbs free energy(ΔG), and entropy (ΔS), respectively (10). All of thermodynamic parameters are given in Table 1.

$$\ln \frac{K_2}{K_1} = \frac{-\Delta H}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \quad (6)$$

$$\Delta G = -RT \ln K \quad (7)$$

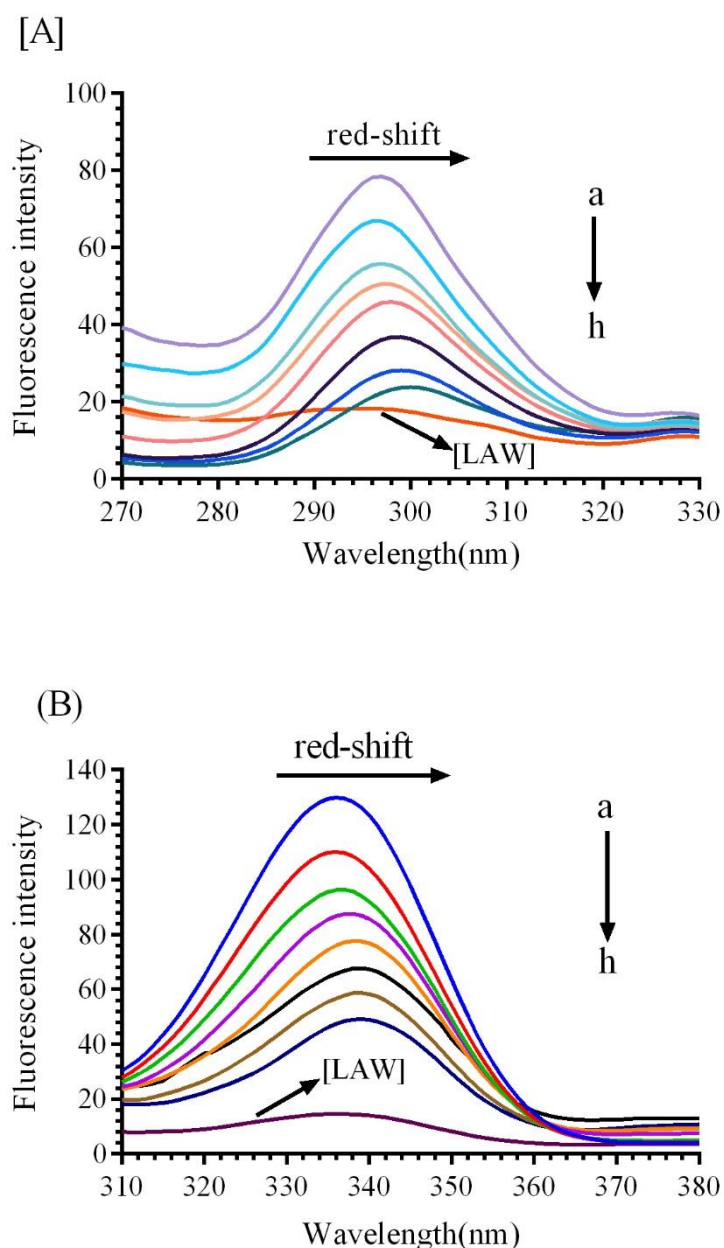


Figure 6. Synchronous fluorescence spectra of BLC in the presence of incremental concentration of LAW (a) 0, (b) 0.47, (c) 4.7, (d) 9.4, (e) 11.8, (f) 14.2, (g) 23.6 and (h) 37.8 μ M, (i) enzyme free LAW (0.7 μ M); (A) $\Delta\lambda=15$ and (B) $\Delta\lambda=60$ nm at 298k.

$$\Delta G = \Delta H - T\Delta S \quad (8)$$

The positive values, obtained from enthalpy changes (ΔH) and the entropy changes (ΔS) reflected that the hydrophobic bindings play a major role in the arrangement of the LAW-CAT complex and the negative quantity of ΔG

illustrated that interaction of LAW with CAT is a spontaneous process (3, 21, 33).

UV-vis spectroscopic studies

UV-vis absorption study can be applied to explore the structural change of BLC upon interaction with LAW. BLC, like most of heme

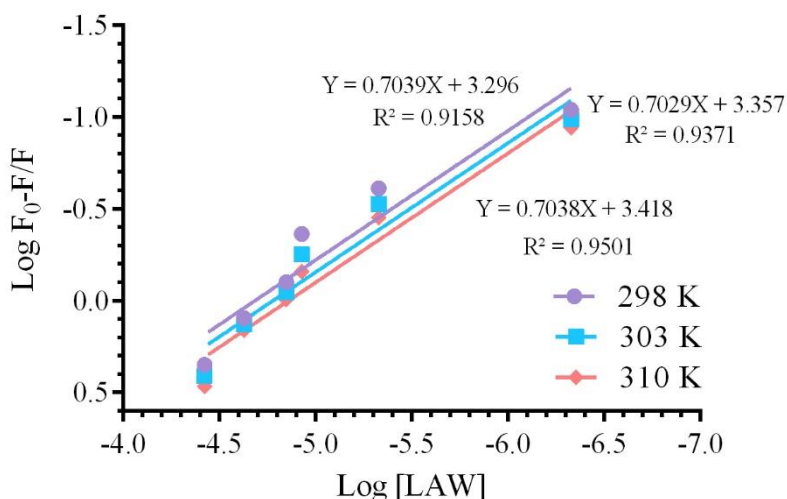


Figure 7. The linear plot of Log F₀-F/F vs. Log [LAW] for quenching of BLC in the presence of LAW at 298, 303 and 310 K.

proteins has two principal absorption area: (1) an absorption band at 280 nm, which is resulted from the aromatic residues including tryptophan (Trp), phenylalanine (Phe), and tyrosine (Tyr) (8,30) and (2) Soret absorption band around 405 nm, which corresponding to $\pi \rightarrow \pi^*$ transfer of electrons in the heme group(11).UV-vis spectra of CAT (1 μM) were monitored in the presence of growing concentrations of LAW (4.6, 9.3 and 18.6 μM) at 298 K (Figure 8). The results indicated that with raising the concentration of LAW, the maximum absorption intensities at 280 nm and 405 nm were enhanced and weakly decreased, respectively. Raising the absorbance at 280 nm shows that the interaction between CAT and LAW leads to change in Trp position and protein conformation. Weak change in the absorbance intensity at 405 nm illustrates a minor structural perturbation in the active site of the CAT.

ATR-FTIR spectroscopy

In order to prove the changes in the secondary structure of the CAT, FT-IR spectroscopic analyses were conducted. The FT-IR spectra of catalase demonstrated three amid bonds (I, II and III). Among the several binding groups of CAT, the amide I in the range of 1600–1700 cm^{-1} (generally caused by

C=O stretching vibration), is more sensitive to conformational variations and is mostly applied to analyze the secondary conformation of the proteins. The absorbance intensity of the amide II band in the range of 1500–1600 cm^{-1} (resulted from C-N stretch vibration combined with N-H bending mode) has been illustrated to be related to the amount of the enzyme-ligand complex. As shown in Figure 9, addition of various concentrations of LAW leads to change in FT-IR spectra of BLC. A shift in amide I from 1637.5 cm^{-1} (for free CAT) to 1635.5 cm^{-1} and 1632.6 for additional concentrations of LAW (9.4 and 23.6 μM), respectively, and reducing the intensity demonstrated the change of the secondary structure and loss of CAT functionality. The amide II, which is located at 1537.2 cm^{-1} had no position shifts, while the intensities were decreased significantly in the LAW-BLC complex, which indicated the reason of maintaining the certain activity of the enzyme. Previous studies have demonstrated that a shift in amide I bands is resulted from various secondary conformational elements: 1620–1645 cm^{-1} from β -sheet, 1645–1652 cm^{-1} for random coils, 1652–1662 cm^{-1} from α -helix, and 1662–1690 cm^{-1} for turns. So LAW mainly changes the secondary structure of β -sheets in BLC structure (27, 34, 35).

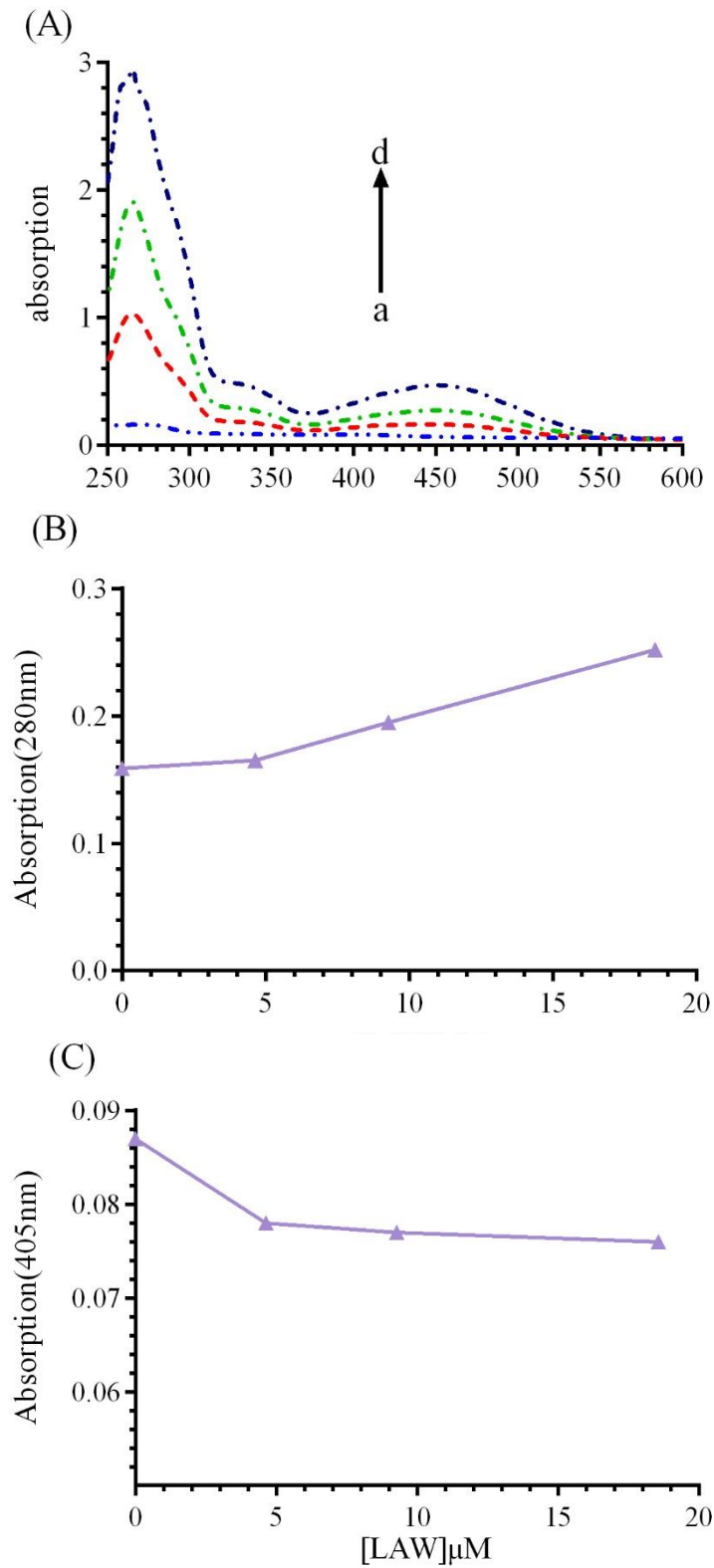


Figure 8. (A) UV-vis spectra of BCL (1 μM) in the absence or presence of different concentrations of LAW; (a) 0, (b) 4.6, (c) 9.3 and (d) 18.6 μM in 50 mM phosphate buffer, pH 7, at 298 K, (B) LAW increases the absorption intensity of BLC at 280 nm, (C) LAW decreases the absorption intensity of BLC at 405 nm.

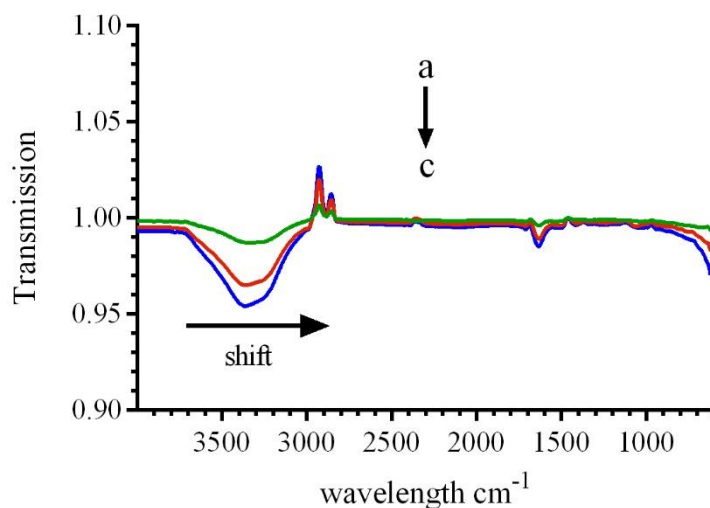


Figure 9. FTIR spectra of BLC (0.7 μM) in the absence or presence of different concentrations of LAW; (a) 0, (b) 9.4, (c) 23.6 in 50 mM phosphate buffer, pH 7, at 298 k.

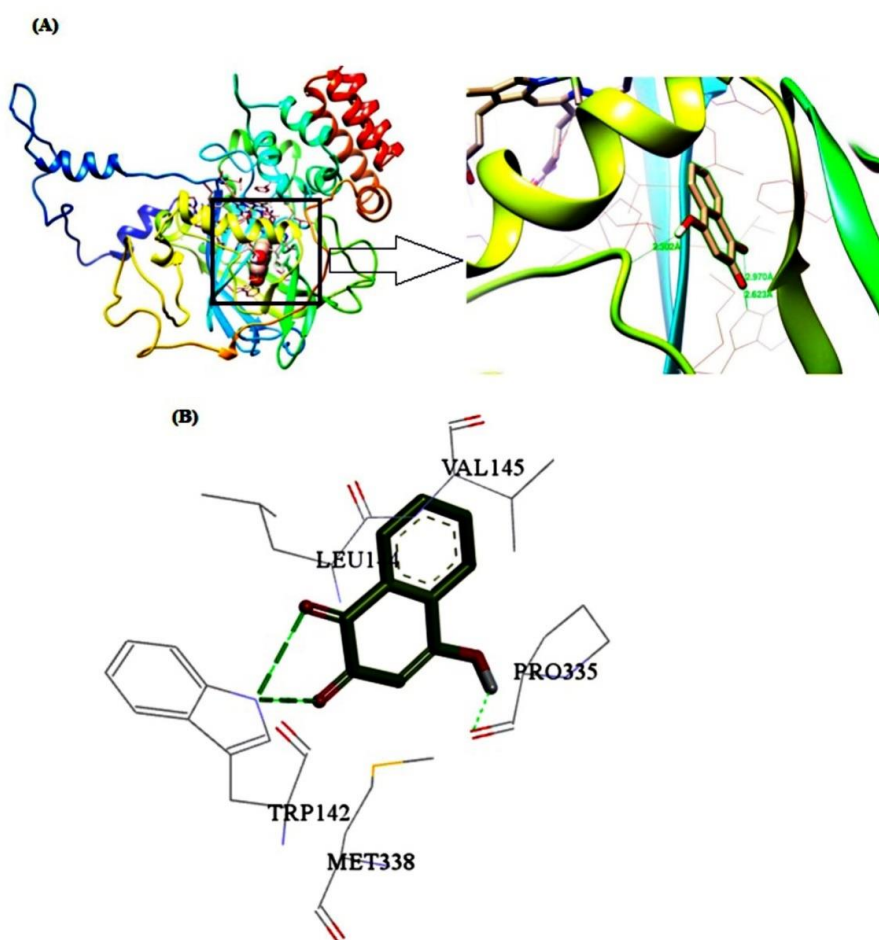


Figure 10. Molecular docking models for BLC-LAW complex. (A) The structure of one subunit of BLC binding site for LAW. (B) Detailed illustration of the binding between BLC and LAW.

Molecular docking studies

To identify the details of the best binding sites and dominant interaction of the ligand-macromolecule complex, the molecular docking computation was carried out based on the minimum energy. According to the obtained results, CAT has one binding site for LAW which is located close to the heme group and between the β -barrel, helical domain and wrapping domain of CAT (Figure 10). Five residues including Trp142, Leu144, Val145, Pro335, and Met338 are involved in the interaction of BLC and LAW. These results are in agreement with synchronous fluorescence data discussed earlier. The driving forces in the interaction between BLC and LAW are composed of hydrophobic and hydrogen bondings, whereas the prior bondings are dominant. The hydrogen bonds were formed between Trp142 and Leu144 as an electron acceptor and electron donor, respectively. The quantity of lowest binding free energy was computed as $-3.85 \text{ kcal mol}^{-1}$ (3, 12, 36). Different values of free energy, obtained from fluorescence and docking simulations may result from various factors such as rigidity of receptor, flexibility of ligand, and effect of buffer solution.

Conclusion

The present study investigated the effect of LAW, as an anti-oxidant and anti-cancer active component of Henna leaves, on the conformation and function of CAT by using various techniques including kinetic study, UV-vis absorption, fluorescence spectroscopy, FT-IR, synchronous fluorescence, and molecular docking approaches. This work indicated that LAW can bind to free enzyme or enzyme-substrate complex through one binding site and changes in the secondary structure and inhibits the activity of CAT by noncompetitive inhibition mechanism. The fluorescence spectroscopic data illustrated that LAW quenches fluorescence intensity of CAT through a dynamic quenching mechanism, and synchronous fluorescence calculations illustrated the alteration in the microenvironment of Tyr and Trp residues by LAW. UV-vis absorption studies revealed that the structure of CAT changes in the presence of

LAW. ATR-FTIR spectroscopic data showed a shift in amide I bands, indicating alterations in the secondary structure of the enzyme. In order to determine the best binding condition between LAW and CAT, the molecular docking studies were performed. Our study may provide valuable information to figure out the importance of LAW in the treatment of the cancer cells through inhibition of catalase, due to high concentration of ROS such as H_2O_2 that could be a therapeutic alternative for the treatment of cancer.

References

- (1) Chelikani P, Carpena X, Fita I and Loewen PC. An electrical potential in the access channel of catalases enhances catalysis. *J. Biol. Chem.* (2003) 278:31290-6.
- (2) Wang Y and Zhang H. Comprehensive studies on the nature of interaction between catalase and SiO_2 nanoparticle. *Mater. Res. Bull.* (2014)60:51-6.
- (3) Rashtbari S, Dehghan G, Yekta R, Jouyban A and Iranshahi M. Effects of Resveratrol on the Structure and Catalytic Function of Bovine Liver catalase (BLC): Spectroscopic and Theoretical Studies. *Adv Pharm. Bull.* (2017) 7:349.
- (4) Harifi-Mood AR, Ghobadi R and Divsalar A. The effect of deep eutectic solvents on catalytic function and structure of bovine liver catalase. *Int. J. Biol. Macromol.*(2017) 95:115-20.
- (5) Sani MF, Kouhsari SM and Moradabadi L. Effects of three medicinal plants extracts in experimental diabetes: Antioxidant enzymes activities and plasma lipids profiles in comparison with metformin. *Iran. J. Pharm. Res.* (2012)11:897.
- (6) Yekta R, Dehghan G, Rashtbari S, Ghadari R and Moosavi-Movahedi AA. The inhibitory effect of farnesiferol C against catalase; Kinetics, interaction mechanism and molecular docking simulation. *Int. J. Biol. Macromol.* (2018) 113:1258-65.
- (7) Panahi Y, Yekta R, Dehghan G, Rashtbari S, Jafari NJ and Moosavi-Movahedi AA. Activation of catalase via co-administration of aspirin and pioglitazone: Experimental and MLSD simulation approaches. *Biochimie*(2019) 156:100-8.
- (8) Chakravarti R, Gupta K, Majors A, Ruple L, Aronica M and Stuehr DJ. Novel insights in mammalian catalase heme maturation: Effect of NO and thioredoxin-1. *Free Radic. Biol. Med.*(2015) 82:105-13.
- (9) Gholamian A, Divsalar A, Saiedifar M, Ghalandari B, Saboury AA and Koohshekan B. Generation of reactive oxygen species via inhibition of liver

- catalase by oxalli-palladium: A spectroscopic and docking study. *Process Biochem.* (2017) 52:165-73.
- (10) Goyal MM and Basak A. Human catalase: looking for complete identity. *Protein Cell*(2010) 1:888-97.
 - (11) Koohshekan B, Divsalar A, Saiedifar M, Saboury AA, Ghalandari B, Gholamian A and Seyedarabi A. Protective effects of aspirin on the function of bovine liver catalase: A spectroscopy and molecular docking study. *J. Mol. Liq.* (2016) 218:8-15.
 - (12) Yekta R, Dehghan G, Rashtbari S, Sheibani N and Moosavi-Movahedi AA. Activation of catalase by pioglitazone: Multiple spectroscopic methods combined with molecular docking studies. *J. Mol. Recognit.* (2017) 30:e2648.
 - (13) Xi X and Zhao D. Maximal point spaces of dcpos satisfying the Lawson condition. *Topol. Appl.* (2017) 230:417-24.
 - (14) de Oliveira AS, Brighente IM, Lund RG, Llanes LC, Nunes RJ, Bretanha LC, Yunes RA, Carvalho PH and Ribeiro JS. Antioxidant and Antifungal Activity of Naphthoquinones Dimeric Derived from Lawsone. *J. Biosci. Med.* (Irvine) (2017) 5:39.
 - (15) Sunassee SN, Veale CG, Shunmoogam-Gounden N, Osoniyi O, Hendricks DT, Caira MR, de la Mare JA, Edkins AL, Pinto AV, da Silva Júnior EN and Davies-Coleman MT. Cytotoxicity of lapachol, β -lapachone and related synthetic 1,4-naphthoquinones against oesophageal cancer cells. *Eur. J. Med. Chem.* (2013) 62:98-110.
 - (16) Bhuiyan MR, Islam A, Ali A and Islam MN. Color and chemical constitution of natural dye henna (*Lawsonia inermis* L) and its application in the coloration of textiles. *J. Clean. Prod.* (2017)167:14-22.
 - (17) Salunke-Gawali S, Kathawate L, Shinde Y, Puranik VG and Weyhermüller T. Single crystal X-ray structure of Lawsone anion: evidence for coordination of alkali metal ions and formation of naphthoquinone radical in basic media. *J. Mol. Struct.* (2012) 1010:38-45.
 - (18) Pryor W. Free radicals in biology: Elsevier(2012).
 - (19) Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* (2007) 39:44-84.
 - (20) Rezaei-Tavirani M, Tadayon R, Mortazavi SA, Medhet A, Namaki S, Kalantari S and Noshinfar E. Fluoxetine competes with cortisol for binding to human serum albumin. *Iran. J. Pharm. Res.* (2012) 11:325-30.
 - (21) Moradi M, Divsalar A, Saboury AA, Ghalandari B and Harifi AR. Inhibitory effects of deferasirox on the structure and function of bovine liver catalase: a spectroscopic and theoretical study. *J. Biomol. Struct. Dyn.* (2015) 33:2255-66.
 - (22) Rashtbari S, Dehghan G, Yekta R and Jouyban A. Investigation of the binding mechanism and inhibition of bovine liver catalase by quercetin: Multi-spectroscopic and computational study. *Bioimpacts*(2017) 7:147.
 - (23) Miri R, Bohlooli F, Razzaghi-Asl N and Ebadi A. Molecular Modeling of Indeno [1, 2-b] Quinoline-9, 11-Diones as Cytotoxic Agents. *Iran. J. Pharm. Res.* (2018) 17:1249-62.
 - (24) Cappiello M, Moschini R, Balestri F, Mura U and Del-Corso A. Basic models for differential inhibition of enzymes. *Biochem. Biophys. Res. Commun.* (2014) 445:556-60.
 - (25) Hu Y and Da L. Insights into the selective binding and toxic mechanism of microcystin to catalase. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* (2014) 121:230-7.
 - (26) Abu Teir MM, Ghithan J, Abu-Taha MI, Darwish SM and Abu-Hadid MM. Spectroscopic approach of the interaction study of ceftriaxone and human serum albumin(2013).
 - (27) Zhao L, Liu J, Guo R, Sun Q, Yang H and Li H. Investigating the interaction mechanism of fluorescent whitening agents to human serum albumin using saturation transfer difference-NMR, multi-spectroscopy, and docking studies. *RSC Adv.* (2017) 7:27796-806.
 - (28) Hassan WE, Eid SM, Kelani KM and Shalaby AA. Spectrophotometric determination and thermodynamic studies of the charge transfer complexes of bambuterol–HCl. *Drug Invention Today*(2013) 5:2-7.
 - (29) Chen L, Zhang J, Zhu Y and Zhang Y. Interaction of chromium (III) or chromium (VI) with catalase and its effect on the structure and function of catalase: An *in-vitro* study. *Food Chem.* (2018) 244:378-85.
 - (30) Zhang G, Ma Y, Wang L, Zhang Y and Zhou J. Multispectroscopic studies on the interaction of maltol, a food additive, with bovine serum albumin. *Food Chem.* (2012) 133:264-70.
 - (31) Kumari M, Maurya JK, Singh UK, Khan AB, Ali M, Singh P and Patel R. Spectroscopic and docking studies on the interaction between pyrrolidinium based ionic liquid and bovine serum albumin. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* (2014) 124:349-56.
 - (32) Shen GF, Liu TT, Wang Q, Jiang M and Shi JH. Spectroscopic and molecular docking studies of binding interaction of gefitinib, lapatinib and sunitinib with bovine serum albumin (BSA). *J. Photochem. Photobiol. B*(2015)153:380-90.
 - (33) Guan J, Dai J, Zhao X, Liu C, Gao C and Liu R.

- Spectroscopic investigations on the interaction between carbon nanotubes and catalase on molecular level. *J. Biochem. Mol. Toxicol.*(2014) 28:211-6.
- (34)Khaskheli AA, Talpur FN, Ashraf MA, Cebeci A, Jawaid S and Afridi HI. Monitoring the *Rhizopus oryzae* lipase catalyzed hydrolysis of castor oil by ATR-FTIR spectroscopy. *J. Mol. Catal. B Enzym.* (2015)113:56-61.
- (35)Glassford SE, Byrne B and Kazarian SG. Recent applications of ATR FTIR spectroscopy and imaging to proteins. *Biochim. Biophys. Acta*(2013) 1834:2849-58.
- (36)Trush MM, Semenyuta IV, Vdovenko SI, Rogalsky SP, Lobko EO and Metelytsia LO. Synthesis, spectroscopic and molecular docking studies of imidazolium and pyridinium based ionic liquids with HSA as potential antimicrobial agents. *J. Mol. Struct.* (2017)1137:692-9.

This article is available online at <http://www.ijpr.ir>
