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Synthesis, Characterization, Antioxidant and ctDNA -Binding Activity of Bi(III)-Quercetin Complex: Multispectral Analysis

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Abstract
Chelation therapy has been used to remove toxic metals from the body for a long time. Flavonoids such as quercetin (QUR), a well-known protective antioxidant and free radical scavenger, can bind to metal cations and protect our bodies from toxic metals. In the current study, we used UV–Vis, ^{1}HNMR, IR, and fluorescence spectroscopic techniques as well as viscosity measurements to investigate the synthesis, characterization, and interaction between Bi(III)-QUR complex and calf thymus DNA (ctDNA) in physiological buffer. The antioxidant activity of the complex was assessed utilizing DPPH and ABTS free radical scavenging, and ferric reducing potential. After chelation of the Bi(III) cation, the antioxidant potential of QUR was reduced. In the presence of ctDNA, the absorption spectrum of Bi(III)-QUR complex was raised, and the fluorescence intensity of Bi(III)-QUR complex was increased. With the addition of the Bi(III)-QUR complex, the relative viscosity of ctDNA rose. These findings indicate that the Bi(III)-QUR complex interacts with ctDNA in a groove-binding mode. The thermodynamic parameters (ΔH, ΔS, and ΔG) of the Bi(III)-QUR complex with ctDNA, as well as well as association constant, K_a, and number of binding sites (n), were assessed from the fluorescence data, indicating that the binding of Bi(III)-QUR complex to ctDNA was primarily driven by hydrophobic interactions.

Keywords
Flavonoids; Chelation Therapy; Bi(III)-Quercetin Complex; Radical Scavenging; Natural Products; Antioxidant.

1. INTRODUCTION

Cancer is one of the world's worst diseases, and despite many efforts to treat it, finding effective therapeutic procedures that are also efficient remains a struggle. Metal-based drugs (metal complexes), particularly when combined with natural products, have made great advances in this area. Metal complexes have had a specific role in medical chemistry and pharmaceuticals since the sixteenth century as medications with a variety of therapeutic qualities. Most of these complexes have been used in the treatment of several diseases such as cancer, leukemia, allergies, and diabetes, anti-inflammatory agents, anti-microbial and antifungal activity, and free radical chain breakers [1-3]. Due to their numerous biological and pharmaceutical properties, as well as their ability to coordinate metal ions and form complexes, healthy clinical, pharmacological, and therapeutic activities of natural compounds such as flavonoids have been extensively studied as templates for the synthesis of novel metal-containing drugs during the drug discovery processes [4,5].

Flavonoids (Fig. 1), also known as bioflavonoids, are natural polyphenolic phytochemicals with a C6-C3-C6 ring structure that are found in a variety of plants, fruits, and vegetables, including seeds, tea, olive oil, and red wine. Plant secondary metabolites are natural chemicals that come in the form of glycosides, acyl glycosides, and esterified forms. They have antiviral, antibacterial, antiallergic, antioxidant, neuroprotective, anti-inflammatory, anticancer, and cardioprotective effects, among other biological actions. Scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals is one of these chemicals' most significant roles, which ensures many of their beneficial effects in the body [4-6].

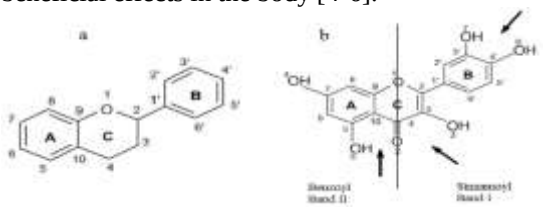

Fig. 1. (a) General structure of flavonoids. (b) Chemical structure of QUR. Divisions of bands I and II related to UV–Vis absorption of Ring A (Band II) benzoyl system; Ring B (Band I) cinnamoyl system in QUR structure.

Due to the presence of carbonyl and 3- or 5-hydroxyl groups in their chemical structure, compounds such as quercetin, chrysin, luteolin, hesperetin, naringenin, apigenin, rutin, kaempferol, morin, and genistein have been reported to have a high binding (chelation) ability to various metal ions, including Ni(II), Cu(II),

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Zn(II), Eu(III), Tb(III), Dy(III), Tm(III), Y(III), Ce(III) and Ge(III). The metal complexes of flavonoids, particularly quercetin (QUR), have been found to treat cancer through several functional pathways. Increased cancer cell cytotoxicity via single-stranded and double-stranded DNA breaks, activation of apoptosis and/or oxidative DNA damage, decreased levels of antiapoptotic proteins, and DNA binding is only a few examples [6-17]. As a result, metal chelation (coordination) by natural chelating ligands affects both the bioavailability of low-abundance metal ions (such as Al(III)) and the toxicity of a variety of toxic metals (such as Pb(II)), as well as flavonoids' antioxidant activity [17].

Bismuth (Bi) is a heavy metal that belongs to the same family as lead, mercury, gold, silver, and arsenic. Although it is generally safe, long-term exposure to bismuth through consuming cosmetics and pharmaceuticals containing Bi(III) can cause negative effects and even poisoning in humans [18]. Bi(III) salts have been used to treat gastrointestinal conditions including gastric and duodenal ulcers, dyspepsia, diarrhea, and colitis [19]. Bi(III) binds tightly to the thiol groups in proteins, enzymes, or tripeptide glutathione and this type of modification may play a crucial role in the function of its targets and transport and delivery of Bi(III) in the biological fluids. Bi(III) attaches to both Zn(II) and Fe(III) sites in proteins and enzymes (e.g. metallothionein and transferring) and is transported throughout the body, causing Bi poisoning symptoms. Absorbing Bi(III) causes neurotoxicity, gastrointestinal toxicity, nephrotoxicity, and hepatotoxicity in the kidneys, lungs, spleen, liver, and brain [20]. Because of their excellent efficiency and minimal toxicity, Bi has been used in medicine for about two centuries. Bi compounds have lately been thoroughly examined for the treatment of several microbiological infections, including syphilis, diarrhea, gastritis, and colitis. Furthermore, the anti-cancer potential of Bi compounds has been studied for decades [21].

The interaction of small molecules (drugs/metal complexes) with DNA has been the subject of extensive research for nearly half a century, and it is an active research area in chemistry, molecular biology, and medicine. It is also a significant feature in pharmacology, leading to the development of more efficient and specifically new targeted drugs with fewer side effects, as well as the in vitro screening of these drugs [13,15,22-30]. The interaction of small molecules with DNA must be investigated to produce effective chemotherapeutic agents and better anticancer treatments. Small molecules can bind to DNA via three possible ways: groove binding, in which ligands bind to nucleotide bases in the major or minor grooves of the DNA through hydrogen-bonding or van der Waals interactions, electrostatic interactions between cationic species and the negatively charged DNA backbone, and intercalative binding, in which aromatic compounds enter between stacked based-pairs and is stronger than two other binding modes [15,23,27,28].

Metal complexes are mostly used as anticancer and antibacterial drugs, so the coordinative complexes between transition/rare-earth metal and flavonoids, particularly quercetin, and nucleic acids (DNA) have gotten a lot of attention in recent years [16,17]. We decided to explore the interaction of the Bi(III)-QUR complex with Calf thymus DNA (ctDNA) because of the physiological actions of metal complexes of flavonoids (ctDNA). It is believed that the findings of this research will aid in the development of novel therapeutic reagents for the treatment of cancer and other disorders.

We prepared a Bi(III)-QUR complex and studied the chelating property of QUR, a common flavonoid, against Bi(III), as well as the change in QUR's antioxidant capabilities following chelation. We also used UV-Vis spectrophotometry, fluorescence spectroscopy, and viscosity measurements to investigate the interaction between the Bi(III)-QUR complex and ctDNA. We believe this will be helpful to understand the binding mechanism and affinity between the Bi(III)-QUR complex and ctDNA, as well as the development of prospective DNA structure and conformation probes.

2. EXPERIMENTAL

2.1. Chemicals and reagents

All reagents and solvents were analytical grades and used without further purification. Extra pure methanol was purchased from Scharlau chemical company (Barcelona, Spain). Quercetin-$2H_2O$, DPPH (2,2-diphenyl-1 picrylhydrazyl), ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt, TPTZ (tripyridyl-s-triazine) purchased from Sigma–Aldrich chemical company. (St. Louis, MO, USA). $Bi(NO_3)_3.5H_2O$ salt and some other chemicals were prepared from the Merck chemical company (Darmstadt, Germany). Stock solution (1.0×10^{-3} M) of Bi(III)-QUR complex was prepared by dissolving an appropriate amounts of complex in double-distilled water.

A stock solution of ctDNA (Sigma Chem. Co., USA) was made by dissolving a sufficient amount of ctDNA in double-distilled water and keeping it at 4 °C. UV absorption at 260 nm with a molar absorption coefficient of ε_{260} = 6600 L $mol^{-1}cm^{-1}$ was used to measure the content of ctDNA in the stock solution [22]. The ratio of 260 nm to 280 nm absorbance was used to determine the purity of the

ctDNA. A ctDNA solution had a UV absorbance ratio of higher than 1.8 at 260 and 280 nm, showing that ctDNA was sufficiently protein-free [29,30].

2.2. Methods and Instrumentation

The absorption spectra for interactions between ctDNA and the Bi(III)-QUR complex were obtained using a UV–Vis spectrophotometer (T60, PG Instruments LTD., Leicestershire, UK) and a 1.0 cm quartz cell. Absorbance measurements were taken in two different ways: i) using a fixed Bi(III)-QUR complex concentration (5.0×10^{-5} M) to which increments of the ctDNA stock solution ($0.5\text{-}2.5)\times10^{-5}$ M were added; ii) using a fixed Bi(III)-QUR complex concentration (5.0×10^{-5} M) to which increments of the ctDNA stock solution (2.5×10^{-6} to 3.5×10^{-5} M) were added. To determine the intrinsic binding constant, K_b, the data were fitted to Eq. (1). [31];

$$[DNA]/(\varepsilon_a-\varepsilon_f)=[DNA]/(\varepsilon_b-\varepsilon_f)+1/k_b(\varepsilon_b-\varepsilon_f) \quad (1)$$

where the apparent, free, and bound Bi(III)-QUR complex extinction coefficients are ε_a, ε_f, and ε_b respectively. Following Beer's law, ε_f was determined using a calibration curve of the isolated Bi(III)-QUR complex in an aqueous solution. A_{obs}/[Bi(III)-QUR] was calculated as the ratio of observed absorbance to Bi(III)-QUR complex concentration. A plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA] yielded a slope of 1/($\varepsilon_b - \varepsilon_f$) and an intercept of 1/$K_b$ ($\varepsilon_b - \varepsilon_f$), where K_b is the slope-to-intercept ratio.

In addition, a UV–Vis spectroscopic analysis of Bi(III)-QUR chelation was carried out by titrating QUR (6.0×10^{-5} M) in methanol solution with various concentrations of $Bi(NO_3)_3.5H_2O$ salt.

A JASCO (FP-750) spectrofluorometer was used for all fluorescence measurements (Tokyo, Japan). Increasing amounts of ctDNA were titrated with fixed amounts of Bi(III)-QUR complex (5.0×10^{-5} M). The fluorescence emission spectra were then measured in the wavelength range of 480–630 nm with an exciting wavelength of 390 nm at different temperatures (298, 305, and 313 K).

A viscometer (Julabo, MD-18V, Germany) was utilized for the viscosity tests, which was kept at a constant temperature of 25±0.1 °C in a thermostatic water bath. The concentration of the Bi(III)-QUR complex was $(0.5\text{-}10)\times10^{-5}$ M, while the concentration of ctDNA was fixed at 5.0×10^{-5} M. The viscosity (η) of the samples was determined using the mean values of three replicated measurements taken with a digital stopwatch. The results were shown as $(\eta/\eta_0)^{1/3}$ vs the [Bi(III)-QUR complex]/[DNA] ratio, where (η_0) represents the viscosity of the DNA solution alone. Viscosity values were determined using the observed flow time of ctDNA-containing solutions (t) and the flow time of buffer solution (t_0), $\eta = (t - t_0)/t_0$ [23,30].

Infrared data collected from the desired complex during synthesis were analyzed using a Bruker Tensor-27 spectrophotometer, and the ^{1}HNMR spectra were recorded using a Bruker 500 MHz spectrometer (Bruker, Rheinstetten, Germany). Chemical shifts were expressed in δ (ppm) units downfield from tetramethylsilane (TMS) as an internal standard.

2.3. Complex formation between Bi(III) and QUR

Using methanol as the reaction medium, the Bi(III)-QUR complex was created through a direct reaction of $Bi(NO_3)_3.5H_2O$ and $QUR.2H_2O$. Solid $QUR.2H_2O$ (0.05 g or 0.008 M) was dissolved in extra pure methanol (20 mL) in a 50 mL two-necked round-bottomed flask fitted with an electromagnetic stirrer and thermometer and covered with foil in 20 minutes, and the color of the solution was pale yellow. After promptly adding $Bi(NO_3)_3.5H_2O$ (0.155g or 0.016 M), the color changed to vivid orange, indicating a very quick reaction between QUR and Bi(III). For 4.0 hours, the solution was agitated. The reaction mixture was then filtered and kept at room temperature to remove the methanol, yielding vivid orange sediment that was the same complex.

2.4. Stoichiometric study of the metal-ligand complex

The continuous variation method was used to define the stoichiometric ratio between QUR and Bi(III) by graphing the absorbance against the mole fraction. In this method, a series of solutions containing varying amounts of the $Bi(NO_3)_3.5H_2O$ and $QUR.2H_2O$ were prepared by mixing of concentration (4.0×10^{-4} M) solutions of both components in different ratios varying from 1:9 to 9:1. Then the absorbance was measured at 430 nm.

2.5. In vitro antioxidant activity of the complex

DPPH, ABTS radical scavenging activity, and ferric reducing antioxidant power (FRAP) were used to assess the antioxidant activity of free QUR and Bi(III)-QUR complex. The DPPH assay was performed using Wettasinghe and Shahidi's method, with significant modifications [17,30,31]. In brief, 100 mL of QUR and the Bi(III)-QUR complex were added to 2.0 mL of DPPH solution (1.0×10^{-2} M in methanol) and the reaction mixture was rapidly agitated. The reduction of DPPH absorbance was monitored by checking at 517 nm for 10 min (A_S). The absorbance of a blank DPPH solution (2.0 mL) was measured at 517 nm as a control (A_C). The following equation was used to compute the percentage of radical scavenging activity (RSA percent), Eq. (2)

$$RSA\ \% = \frac{100(A_C - A_S)}{A_C} \quad (2)$$

The antioxidant capacity of most synthetic and natural antioxidants, such as flavonoids, is linked to the reduction of metal ions such as Fe^{3+}. To test the capacity of the Bi(III)-QUR complex in metal reduction and compare it to the natural ability of the QUR, the FRAP assay was employed, with some changes, according to Benzie and Strain [32]. In this method the light blue Fe(III)–TPTZ solution changes to the dark blue one, in the presence of antioxidants, which confirms the production of Fe(II)–TPTZ in the solution. The FRAP reagent was freshly prepared by mixing 2.5 mL of solutions TPTZ (1.0×10^{-2} M, in 4.0×10^{-2} M HCl) and $FeCl_3$ (2.0×10^{-2} M) in 25 mL of acetate buffer (1.0×10^{-1} M). These changes were linked to an increase in absorbance measured at 593 nm for different concentrations of the QUR and Bi(III)-QUR complex in FRAP reagent [(5, 10, 15, 20, 30 and 40)$\times10^{-3}$ M]. The FRAP values for both the QUR and Bi(III)-QUR complexes were determined using a standard calibration curve based on varied $FeSO_4.7H_2O$ concentrations.

The ABTS radical scavenging activity was based on Pennycooke, Cox, and Stushnoff's technique [33]. For the creation of green-colored $ABTS^+$, ABTS powder (54.2 mg) was dissolved in 10 mL of phosphate buffer (5.0×10^{-3} M, pH 7.0) and combined with 1.0 g of MnO_2 and incubated at room temperature for 30 minutes. After centrifugation for 5.0 minutes and filtration, the filtrate was diluted with phosphate buffer until the absorbance of the solution equals with 0.70 ± 0.01 in 723 nm. Different quantities of QUR and Bi(III)-QUR complex [(1.0-7.0)$\times10^{-4}$ M] were combined with 2.0 mL of ABTS solution and incubated at room temperature for 10 minutes. Consequently, the absorbance was measured at 723 nm. The following equation was used to compute the percentage of radical inhibition activity, Eq. (3)

$$A_{723nm}(\%) = \frac{(1 - A_f)}{A_o} \times 100 \quad (3)$$

where A_o is the absorbance of the unrestrained ABTS radicals and A_f is the absorbance after 10 min following the addition of the complex.

3. RESULT AND DISCUSSION

3.1. Synthesis and characterization of Bi(III)-QUR complex

3.1.1. UV–Vis spectroscopic study

The UV–Vis absorbance spectra of QUR and Bi(III)-QUR complex in methanol solution, (with increasing concentration) are shown in Fig. 2. QUR shows two distinct absorption bands In the UV–Vis range. Band I (cinnamoyl) in 300–400 nm, and band II (benzoyl) in 240–300 nm wavelength ranges, respectively (Fig. 1a and 1b) [34]. Band I gradually changed to a longer wavelengths as Bi(III) solution was added, accompanied by a modest decrease in the absorption. Simultaneously, a second, stronger absorbance peak showed at 430 nm, indicating that a complex between QUR and Bi(III) has formed [17, 29,30,34]. Bi(III) has joined to the 3-hydroxyl and 4-carbonyl of ring C, as evidenced by the new signal at 430 nm [17]. The interaction of Bi(III) with the 3-hydroxyl group of QUR causes electron redistribution between QUR and Bi(III), resulting in the formation of an extended bonding system. On the other hand, complex formation lead to an increase in the band I absorption peak, which is due to chromophore structural alterations in the benzoyl system. At 392 nm, an isosbestic point was presented, which is revealing the creation of a complex [17,29].

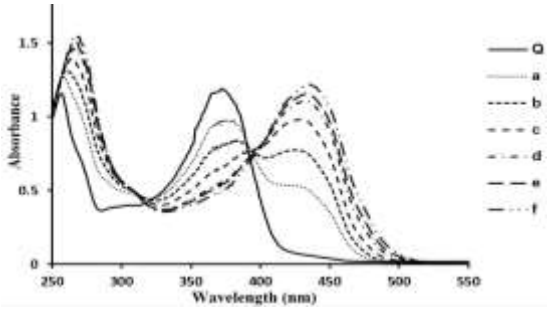

Fig. 2. UV–Vis absorption spectra of free QUR (6.0×10^{-5} M) and Bi(III)- QUR mixtures at different concentrations of Bi(III): (a) 3.0×10^{-5} M ,(b) 6.0×10^{-5} M, (c)12×10^{-5},(d) 18×10^{-5} M,(e) 24×10^{-5} M and (f) 3.0×10^{-4} M in methanol solution.

The stoichiometric composition of the chelate was validated using Job's approach (The continuous variation method). With the addition of $Bi(NO_3)_3.5H_2O$, the absorbance band of QUR exhibited at 360 nm and a new characteristic band of the complex was observed at 430 nm. The absorbance plots at 430 nm (Fig. 3) against the molar fraction of QUR (X) has a maximum absorbance at X_L = 0.5, confirming 1:1 stoichiometric ratio for the complexation of Bi(III) and QUR.

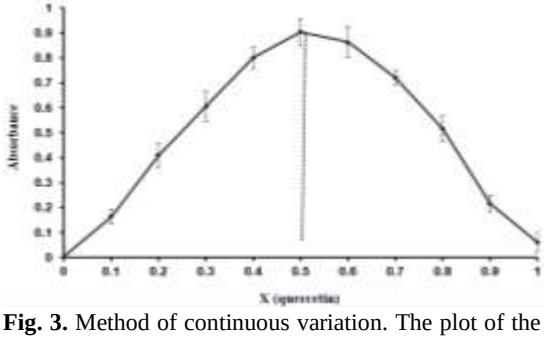

Fig. 3. Method of continuous variation. The plot of the absorbance at 430 nm (λ_{max} of the complex) versus mole fraction of QUR.

Table1. Assignment of the IR spectra of the QUR and the Bi(III)- QUR complex (band position in cm-^{1}).

Compound	γ(O-H)	γ(C=O)	γ(C=C)	γ(C-OH)	γ(C-O-C)	γ(O-Bi)
Quercetin	3408.1	1666.4	1611	1319.1	1262.6	-
Complex	3446.81	1646.57	1601.06	1359.85	1266.52	427.32

3.1.2. Infrared spectral study

IR spectroscopy was used to determine the coordination sites and binding characteristics of QUR. The major bands with tentative assignments are listed in Table 1. By comparing the IR spectra of QUR with the Bi(III)-QUR complex, important information can be achieved. The C=O stretching mode occurs at 1664 cm^{-1} of free QUR, however, which has been shifted to 1645.6 cm^{-1} by the formation of complex, which and indicates incorporation of C=O oxygen with Bi cation [35]. The frequencies ν(C=C) and (C–O–C) are related to the bands at 1611 cm^{-1} and 1262 cm^{-1}, which are slightly displaced by the complex formation. Furthermore, a shift in the frequency of the infrared band (1319.1 cm^{-1} to 1341.9 cm^{-1}) indicates the deformation mode of the ν(C–OH), and the coordination of the ortho-dihydroxy group in the quercetin B ring (3-OH) with Bi cations. The existence of a peak at 427.3 cm^{-1} denotes the creation of a (O–Bi) bond during complex formation. A wide OH-stretching transition bond (3408.1 cm^{-1} to 3446.8 cm^{-1}) specifies the presence of water molecules in the complex structure [36].

3.1.3. ^{1}H NMR spectroscopy

^{1}H NMR spectroscopic analysis of free QUR and Bi(III)-QUR complex was done in DMSO solution and the main data are presented here: QUR; δ6.18 (1H, H-6); δ6.40 (1H, H-8); δ6.89 (1H, H-5′); δ7.54 (1H, H-6′); δ7.67 (1H, H-2′); δ9.29 (1H, 3′-OH); δ9.39 (1H, 4′-OH); δ9.6 (1H, 3-OH); δ10.8 (1H, 7-OH); δ12.49 (1H, 5-OH). Bi(III)-QUR complex; δ6.3(1H, H-6); δ6.6 (1H, H-8); δ6.9 (1H, H-5′); δ7.57 (1H, H-′6); δ7.99 (1H, H-2′); δ9.2 (1H, 3′-OH); δ9.22 (1H, 4′-OH); δ 9.8 (1H, 3-OH); δ10.95 (1H, 7-OH).

^{1}H NMR data indicate that 5-OH protons were deleted from the structure of QUR after complexation with Bi(III); as well as UV-Vis and IR data confirmed the structural changes of QUR following the coordination of Bi cations through 5-OH and 4-oxo groups in A–ring or 3-OH and 4-oxo groups in C-ring or the 3′, 4′-dihydroxyl (catechol) chelation sites.

3.2. Radical scavenging potential of QUR and Bi(III)- QUR complex

QUR is a potent antioxidant and radical-scavenging, as well as metal chelating natural polyphenol compound. The ability of QUR to scavenge oxidants and free radicals is altered when metal cations get complexed [6,17]. Because of its spare electron delocalization across the whole molecule, DPPH is a stable free radical and its solution is a dark violet color with a maximum wavelength of 517 nm. When a DPPH solution is combined with a substrate that acts as a hydrogen donor, a stable non-radical form of DPPH is formed, along with a shift in color from violet to pale yellow. QUR adds two hydrogen atoms to DPPH and is converted into a quinone intermediate [17]. Fig. 4 (a) shows the DPPH scavenging activity of various concentrations of QUR and the Bi(III)-QUR complex. The QUR has a higher free radical scavenging activity than the Bi(III)-QUR complex.

We also employed the ABTS scavenging assay to confirm the anti-radical capability of synthesized complex. Fig. 4 (b) shows the impact of QUR and the QUR–Bi (III) complex on the ABTS radical. When an antioxidant is added to the $ABTS^{\cdot+}$ radical blue-green media, the radical is reduced to its non-radical (ABTS) form and becomes colorless [17,29].

$$ABTS^{\cdot+} + AH \longrightarrow ABTS^{+} + A \qquad \text{Eq. (4)}$$

Gradual increase in the concentrations of both QUR and Bi(III)-QUR complex, lead to a decrease in absorption peak of ABTS at 723 nm. Considering scavenging capacities on both ABTS and DPPH radical, it was shown that free QUR's scavenging potential decreases following the complex formation with Bi(III).

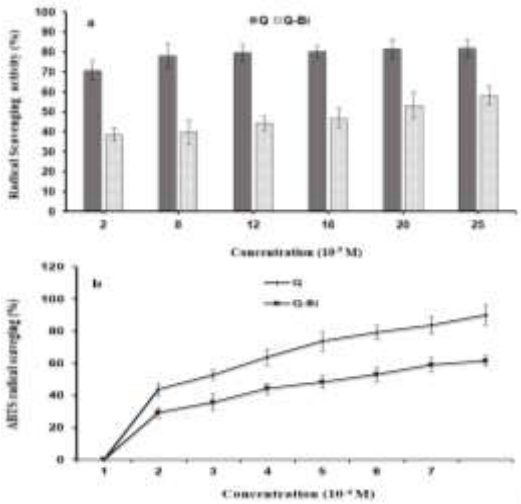

Fig. 4. (a) DPPH radical scavenging (%) and (b) ABTS radical scavenging (%) of different concentrations of QUR and the Bi(III)- QUR complex. (a) [(2, 8, 12, 16×, 20 and 25×)10^{-5} M] and (b) [(1.0-7.0)×10^{-4} M].

3.3. Ferric reducing the activity of QUR and Bi(III)-QUR complex

Metal-induced oxidative stress may protect by the chelating potential of natural antioxidants and lowering their concentration in the media. The FRAP method can be used to determine the reduced ability of antioxidants like flavonoids. The FRAP assay relies on the reducing ability of antioxidants and converting Fe^{3+} to Fe^{2+} coordinated with TPTZ. The reduction in absorbance is related to the concentration of antioxidants [37]. The FRAP data obtained from the ferric reducing activity of QUR and Bi(III)-QUR complex are shown in Fig. 5, confirming the complex's lower antioxidant power compared to free QUR. These results show that QUR and the Bi(III)-QUR complex can donate electrons and so react with free radicals or stop chain reactions, but metal chelation reduces the redox potential of QUR and decreases the electron transfer process to the radical agent.

Flavonoids' antioxidant properties are closely linked to their chemical structure. Phenolic compounds represent their antioxidant effects by two fundamental methods; hydrogen atom transfer and electron donation. The greater antioxidant potential of free QUR in comparison with Bi(III)-QUR complex may be attributed to the significant contribution of the hydroxyl groups on QUR structure, and replacing hydrogen atoms by Bi(III) reduces their hydrogen donation and radical-scavenging abilities. This confirms that metal ions have a substantial impact on chemical structure and antioxidant potential of QUR.

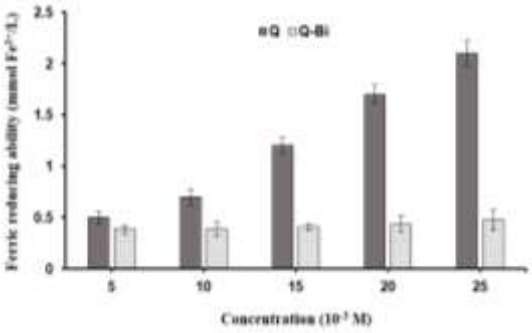

Fig. 5. Ferric reducing antioxidant power ($mmolFe^{2+}/L$) of QUR and Bi(III)- QUR complex in different concentration; [(5, 10, 15, 20 and 25)×10^{-3} M].

3.4. Interaction between Bi(III)-QUR complex and ctDNA

3.4.1. Molecular absorption spectroscopy

UV-Vis absorption spectrophotometry is possibly the most common and widely used instrumental approach for evaluating small molecule binding to DNA. The spectroscopic features of DNA that rely on its double-helical structure are known as "hyperchromic" and "hypochromic" effects. Hypochromism results from the DNA contraction in the helix axis, as well as conformational changes, in contrast hyperchromism results from unwinding or damage to the double-helix structure of DNA. When compounds bind to DNA via intercalation, the result is usually hypochromism and red shift (bathochromic shift), whereas the hyperchromic effect is caused by the electrostatic interaction between the positively charged compounds and DNA double-helix. This type of change is maybe due to the perturbation and uncoiling in the secondary structure of DNA following the contact with exogenous compounds [23,29,30].

As shown in Fig. 6 (a), the absorption spectra of ctDNA (the band at 260 nm of DNA that appears due to the π–π^* transitions of DNA bases) rose as the Bi(III)-QUR complex concentration was raised,. This is a common hyperchromic phenomenon. As a result, these findings suggest that when the Bi(III)-QUR complex binds to DNA, it damages the double-helical structure.

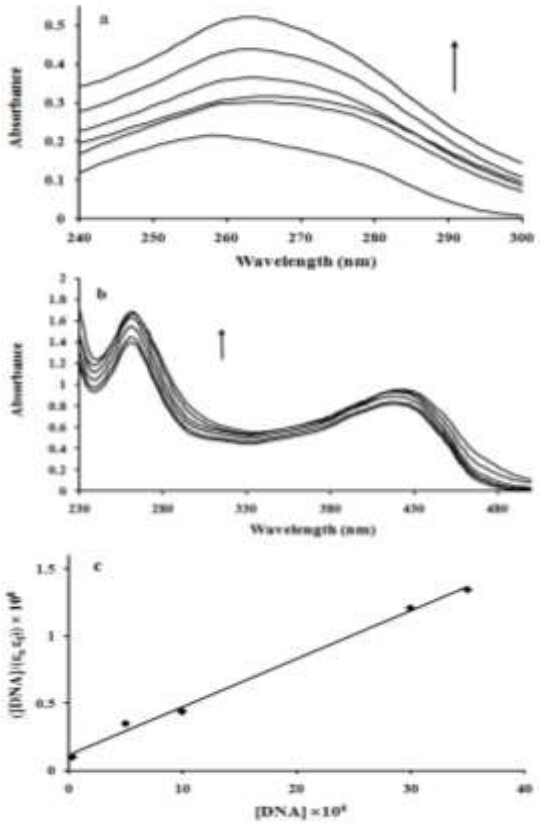

Fig. 6. Absorption spectra: (a) ctDNA (5.0×10^{-5} M) in the absence (top spectrum) and presence of increasing amount of Bi(III)- QUR,([Bi(III)- QUR]/[ctDNA] = 0.1, 0.2, 0.3, 0.4 and 0.5; subsequent spectra. Arrow shows the absorbance changes upon increasing Bi(III)- QUR concentration; (b) Bi(III)- QUR complex (5.0×10^{-5} M) in the absence and presence of increasing amounts of ctDNA, [ctDNA]/[Bi(III)- QUR] = 0.0, 0.05, 0.1, 0.2, 0.6 and 0.7. Arrow shows the absorbance changes upon increasing ctDNA concentration. (c) [ctDNA]/(ε_a - ε_f) versus [ctDNA] plot.

The absorption spectra of the Bi(III)-QUR complex following the addition of various amounts of ctDNA are shown in Fig. 6 (b). There is a strong stacking behavior between aromatic and planar molecules and DNA double-helix in the intercalative mode of interaction and leads to hypochromism and red shift in DNA absorption spectrum [23]. By increasing the amount of ctDNA into Bi(III)-QUR complex solution, the absorption bands show a clear hyperchromism trend, demonstrating that the interaction of Bi(III)-QUR complex with ctDNA could take place in a non-intercalative fashion.

Furthermore, the binding constants (K_b) for the Bi(III)-QUR complex with ctDNA were estimated to be 1.0×10^5 M^{-1} (Fig. 6 (c)), which was lower than the binding constants of classical intercalators, which were on the order of 10^6–10^7 [28,38,39].

3.4.2. Fluorescence spectroscopic studies

Fluorescence measurements can reveal details on how small molecule compounds attach to biomolecules, such as the binding mechanism, drug localization and manner of interaction with DNA, binding constants, binding sites, and ligand orientation and proximity to DNA. Intercalating molecules could insert into the base stacks and cause a significant increase in fluorescence emission intensity of DNA double-helix, whereas hydrogen bonding and electrostatic or hydrophobic interaction between groove binding agents and sugar-phosphate backbone lead to a decrease in the fluorescence spectral intensity of DNA molecule [6,23,40,41]. Since Bi(III)-QUR is a fluorescent complex, direct fluorescence emission method was used to investigate the interaction mode between Bi(III)-QUR complex and ctDNA.

Bi(III)-QUR complex displays a good fluorescence spectrum with maximal wavelengths range of 480-640 nm. The change in the emission spectra of Bi(III)-QUR complex following the interaction with increasing concentrations of ctDNA is shown in Fig. 7. When the amount of DNA added to the complex solution is increased, the intensity of the emission increases, indicating a successful interaction between DNA and Bi(III)-QUR complex.

The binding constants between DNA and ligand were calculated using fluorescence data according to the previously published researches [42]. Considering that there is a 1:1 stoichiometry in Bi(III)-QUR complex, the binding constant (K_a) and number of binding sites (n) calculated by Eq. (5)

$$(F_0 - F)/(F - F_\infty) = K_a \times [DNA] \quad (5)$$

The binding constant K_a was obtained by plotting $\log (F_0 - F)/(F - F_\infty)$ versus log [DNA], where F_0 and F_∞ are the relative fluorescence intensities of Bi(III)-QUR complex alone and Bi(III)-QUR complex in a saturated ctDNA solution. The number of analogous binding sites (n) is the slope of the double-logarithm plot (Fig. 8) derived from experimental data, whereas the value of log [QUR] at log=0 equals the negative logarithm (pKa) of the association constant (K_a). Table 2 shows the binding constants (K_a) for the Bi(III)-QUR complex and ctDNA and the number of Bi(III)-QUR binding sites on the ctDNA molecule. The number of binding sites (n) of the ctDNA molecule in 298 K is near to unit, and n varies with temperature, indicating that the accessibility of the Bi(III)-QUR complex to the ctDNA molecule changes with temperature.

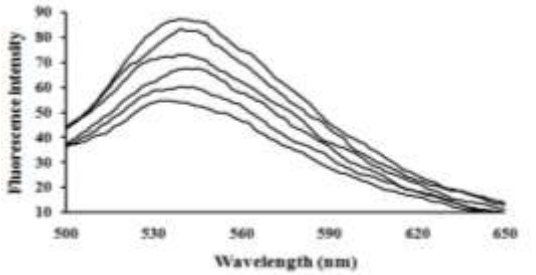

Fig. 7. Fluorescence emission spectra of Bi(III)- QUR complex (5.0×10^{-5} M) in the absence and presence of increasing amounts of ctDNA, ([DNA]/[complex] = 0.0, 0.05, 0.08, 0.3, 0.9 and 1.0.

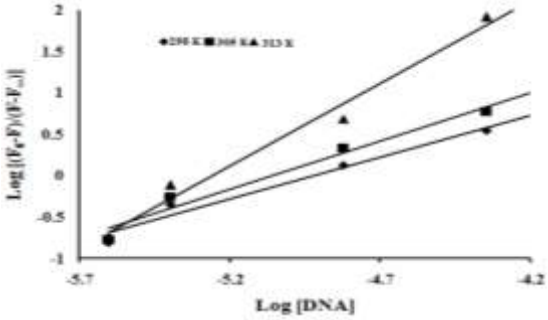

Fig. 8. Variation of log [(F_0-F)/(F-F_∞)] with a log of the concentration of ctDNA.

Table 2. The binding constants K_a and thermodynamic parameters between Bi(III)- QUR and DNA at different temperatures.

Temperature (K)	K_a ($\times10^4$) M^{-1}	n	ΔG (KJ/mol)	ΔH (KJ/mol)	ΔS (J/mol/K)
298	8.4	1.0	-28.0	+39.9	+227.7
305	10.1	1.17	-29.6		
313	10.8	1.99	-31.4		

3.4.3. Thermodynamic parameters

The formation of a complex between ligands and macromolecules takes place in a thermodynamic process and is dependent on temperature changes. Weak molecular interaction driving forces, including hydrophobic and van der Waals forces, hydrogen bonds, and electrostatic interactions play the main role in the interaction between small ligands and macromolecules. The major evidence for proving the binding force is the thermodynamic characteristics of the binding reaction. According to Ross [43,44], the main forces involved in the interaction processes can be determined by evaluating thermodynamic parameters, including enthalpy change (ΔH) and entropy change (ΔS). ΔH>0 and ΔS>0 indicate hydrophobic interaction from the thermodynamic standpoint; the van der Waals forces can be estimated from a thermodynamic condition in which ΔH<0 and ΔS<0; and finally ΔH<0 and S>0 suggest electrostatic forces as driving forces during the interaction. The ΔH can be seen as a constant if the temperature is only slightly changed. The van't Hoff equation can then be used to determine its value and that of ΔS, Eq (6):

$$\log K_a = \frac{\Delta H}{2.303RT} + \frac{\Delta S}{2.303R} \quad (6)$$

$$\Delta G = \Delta H - T\Delta S \quad (7)$$

where R is the gas constant and K_a is the binding constant at the appropriate temperature. Temperatures of 298, 305, and 313 K were employed. The slope and intercept of the linear van't Hoff plot based on log K_a versus 1/T were used to calculate the enthalpy change (ΔH) and entropy change (ΔS). The change in free energy (ΔG) was calculated using Eq (7). Table 2 summarizes the thermodynamic parameters for the interaction of the Bi(III)-QUR complex with ctDNA. Because of the strong association between complex and DNA, the standard free energy changes (ΔG) are large and negative, as shown in the Table. The negative value of ΔG revealed that the contact is spontaneous, however the positive ΔH and ΔS values associated with the interaction of Bi(III)-QUR complex, revealed that the binding is primarily entropy-driven and the enthalpy is unfavorable for it. In other words, hydrophobic contact is crucial in the binding process.

3.5. Viscosity Measurements

Changes in the viscosity of ctDNA in the presence of incremental amounts of the Bi(III)-QUR complex were measured to further investigate the binding parameters. According to the conventional intercalation model, small planar ligands accommodate into separated base pairs which leads to lengthening the double-helix DNA and an increase in DNA viscosity [17,45]. A partial and/or non-classical intercalator, on the other hand, can bend (or kink) the double-helix DNA axis, by decreasing its effective length and reducing its viscosity. Ligands that exclusively bind to DNA minor or major grooves, typically can cause little or no changes in DNA viscosity [45]. The effect of the Bi(III)-QUR complex on the relative viscosity of ctDNA is shown in Fig. 9. Our data show that the Bi(III)-QUR complex enhances the relative viscosity of ctDNA in a concentration-dependent manner. Viscosity results imply that the bridged structure between Bi(III)-QUR complex and ctDNA promotes duplex extension. Binding may take place by inner-sphere complex formation via phosphate oxygen, indicating that Bi(III)-QUR complex binds to surface of DNA molecule or forms bridged adducts with double-helix, according to Liu et al. [46].

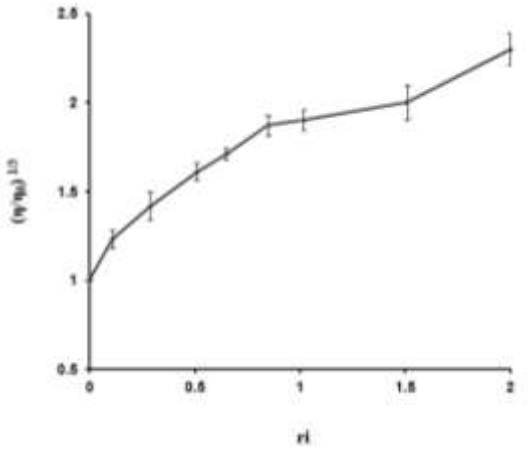

Fig. 9. Effect of increasing amounts of complex on the viscosity of ctDNA (5.0×10^{-5}M) in 0.1 M Tris–HCl buffer, ([Bi(III)- QUR)]/[ctDNA] = 0.0, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.0.

4. CONCLUSION

The interaction of QUR with Bi cations (Bi(III)) was investigated in this study. The UV–Vis spectra of QUR in MeOH show two main absorption bands at 256 and 372 nm; however, after complexation with Bi(III), the λ_{max} was shifted to a higher wavelength and a new band at 430 nm appeared. The stoichiometric composition of the complex was validated using Job's approach. The complex's stoichiometric composition has been determined to be 1:1 (L/M). Spectroscopic evidence suggests that the QUR molecule can chelate Bi(III) cations via chelation sites such as 5-hydroxy-4-carbonyl, 3-hydroxyl-4-carbonyl, or 3′,4′-dihydroxyl (catechol). The results of the DPPH, FRAP, and ABTS methods revealed that both QUR and QUR–Bi(III) complex could react with free radicals and terminate chain reactions by donating an electron or a hydrogen atom, in a time and dose-dependent manner and chelating metal ions lower the redox potential of QUR molecule.

UV–Vis and fluorescence spectroscopic techniques, as well as viscosity measurements, were used to demonstrate the binding interactions of the Bi(III)-QUR complex with ctDNA in a physiological buffer. The thermodynamic parameters of the Bi(III)-QUR complex with ctDNA were calculated and the binding constants of the Bi(III)-QUR complex with ctDNA were measured at different temperatures. The results showed that the Bi(III)-QUR complex may attach to ctDNA, with groove binding, being the most common mechanism of binding. The intrinsic binding constant ($K_b = 1.0\times10^5$ M^{-1}) is closer to that of DNA groove binding. Hydrophobic contact was estimated to be important in the binding of the QUR-Bi(III) complex to ctDNA. The binding manner of the Bi(III)-QUR complex with ctDNA reported here, we believe, will provide significant information on the mechanism of anticancer medications binding to DNA, which will aid in the development of novel therapeutic agents.

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سنتز، مشخصه‌یابی و بررسی فعالیت آنتی‌اکسیدان و اتصال به DNA دو رشته کمپلکس کرستین-بیسموت (III): مطالعات اسپکتروسکوپی

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چکیده

شلات درمانی از دیرباز برای حذف فلزات سمی از بدن مورد استفاده قرار می‌گرفته است. فلاونوئیدهایی مانند کوئرستین (QUR)، یک آنتی اکسیدان محافظ شناخته شده و بعنوان پاک‌کننده رادیکال‌های آزاد، می‌توانند به کاتیون‌های فلزی متصل شده و از بدن ما در برابر فلزات سمی محافظت کنند. در مطالعه حاضر، از تکنیک‌های طیف‌سنجی ماوراء بنفش-مرئی (UV-vis)، 1HNMR، IR و فلوئورسانس و همچنین اندازه‌گیری‌های ویسکوزیته برای سنتز، مشخصه یابی و مطالعه برهم‌کنش بین کمپلکس Bi(III)-QUR و DNA تیموس گوساله (ctDNA) در بافر فیزیولوژیکی استفاده شد. فعالیت آنتی اکسیدانی کمپلکس با استفاده از مهار رادیکال آزاد DPPH و ABTS و پتانسیل کاهش آهن (III) مورد ارزیابی قرار گرفت. پس از شلاسیون کاتیون بیسموت، Bi(III)، پتانسیل آنتی‌اکسیدانی QUR کاهش یافت. در حضور ctDNA، طیف جذب کمپلکس Bi(III)-QUR و شدت فلوئورسانس کمپلکس Bi(III)-QUR افزایش یافت. همچنین با افزودن کمپلکس Bi(III)-QUR، ویسکوزیته نسبی ctDNA افزایش یافت. این یافته‌ها نشان می‌دهد که کمپلکس Bi (III)-QUR با ctDNA در حالت اتصال به شیار برهم کنش می‌دهد. پارامترهای ترمودینامیکی (ΔH، ΔS و ΔG) کمپلکس Bi(III)-QUR با ctDNA، و همچنین ثابت اتصال، Ka، و تعداد مکان‌های اتصال (n)، از داده‌های فلوئورسانس ارزیابی شدند، و نتایج نشان داد که اتصال کمپلکس Bi(III)-QUR به ctDNA عمدتا بواسطه نیروهای آبگریز پیش می‌رود.

واژه‌های کلیدی

فلاونوئیدها؛ شلات درمانی؛ کمپلکس Bi(III)-QUR؛ پاکسازی رادیکال؛ محصولات طبیعی؛ آنتی‌اکسیدان.