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Spectroscopic Studies on the Interaction of Aloe-Emodin and DNA

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In this paper, DNA intercalation theory is introduced to study the interaction and the binding mode of aloe-emodin and DNA by fluorescence and resonance light scattering spectroscopy. Meanwhile, from the perspective of DNA intercalator we investigate pharmacological action of aloe-emodin and the effect of DNA and aloe-emodin on different conditions by spectrum methods. The work shows that the interactions of aloe-emodin and DNA result in the endogenous fluorescence quenching of aloe-emodin,which belongs to a static quenching mechanism. The binding constant and the binding site constant of the static quenching were calculated as 5.765×10^2 L mol and 0.7127. The result also indicates that four enhanced resonance light-scattering peaks appear at 240, 308, 468 and 557 nm, respectively. The resonance light-scattering peaks at 557 nm, which intensity enhances with the addition of DNA, was selected as investigation. This phenomenon were similar to ethidium bromide (EB) interaction of DNA, therefore aloe-emodin combines with DNA by intercalation. The interaction of aloe-emodin and DNA was influenced by different solution conditions. Resonance light-scattering intensity enhances on low pH value, sodium chloride, glucose and polar amino acids. Contrarily, nonpolar amino acids decrease the resonance light-scattering intensity. The alteration in environmental factors has a significant change in fluorescence intensity of aloe-emodin-DNA system. Therefore, the changing environmental factors can affect the interaction between aloe-emodin and DNA, so as to achieve the purposes of attenuation and synergistic effect.

Key Words: Aloe-emodin, DNA intercalator, Fluorescence spectroscopy, Resonance light scattering.

INTRODUCTION

DNA is the important target point of many medicine molecules, especially the anticancer drugs. The small medicine molecules could locate and combine with the bigger molecules of DNA specifically and then influence the gene's ability of regulation and expression to get the treatment effect. However, the inappropriate use of medicine could cause the damage of normal DNA and have the toxic side effects. Lerman[1] first proposed the model of DNA intercalator which is a large class of planar polycyclic aromatic molecules has been found to have the ability to intercalate into DNA in the space between two adjacent base pairs. Planar polycyclic aromatic molecules (DNA intercalator) are able to, interacte with target DNA, form DNA intercalator-DNA with π-π interaction, van der Waals force, hydrogen bond, hydrophobic interactions, charge transfer, molecular orbital interactions[2-4]. The act of intercalation induces local structural changes to the DNA, including unwinding of the double helix and lengthening of the DNA strand. Such a structural change may lead to DNA transcription and replication is delayed or inhibited, thus demonstrating the cytotoxicity and antitumor antiviral activity[5]. The aloe-emodin

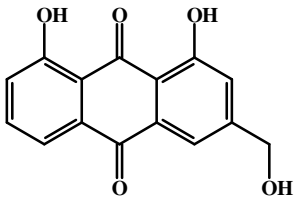

Fig. 1. Structure of aloe-emodin (AE)

(Fig. 1), which has the function of antitumor, antifungus, antivirus, defecation, purgation and so on, is the important anthraquinone component of aloe[6]. However, during the process of clinical use of medicine containing aloe-emodin, some reports of large intestine melanism, acute renal failure and some other untoward effects arose[7]. It is possible that the DNA damage caused by the interaction of aloe-emodin molecule and DNA is the reason of these untoward effects. In this paper, fluorescence and resonance scattering spectra was used in researching the interaction mechanism of aloe-emodin and DNA. At the same time, combination of more mature DNA intercalator theory which study on antitumor drugs, trying to study the pharmacological effects of drug and it's effect *in*

vivo by means of spectroscopy. Based on this, further study on how to avoid the toxic and side effects of Chinese herbal, provide reference to construction related herbs compatibility to reduce its toxicity.

EXPERIMENTAL

A Shimadzu RF-5301PC fluorophotometer (Kyoto, Japan) with a quartz cell of 1 cm path length is used to measure the fluorescence. Absorption spectrum was measured by using a UNICO UV-2102 UV-vis spectrophotometer (Shanghai) and quartz cells (1 cm × 1 cm) are employed in the determination.

Natural double DNA used in this study includes herring sperm DNA (fs DNA, Sigma). The stock solutions were prepared by dissolving the solid DNA in doubly distilled water with occasionally gentle shaking,and stored at 4 ºC. The concentration of DNA solution was determined by UV absorption at 260 nm using the molar absorption coefficient ε_{260} = 6600 mol^{-1} cm^{-1}. Concentration of DNA in the stock solution was 3.78×10^{-4} mol L^{-1} in the experiment.

The stock solution of 1.0×10^{-4} mol L^{-1} aloe-emodin (National Institute for Control of Pharmaceutical and Biological Products) was prepared by ethanol solutions. A Britton-Robinson buffer (pH 7.40) was used to control the pH of the reaction system. All reagents were of analytical reagent grade and doubly distilled water was used throughout the experiments.

Fluorescence spectrum of aloe-emodin interacting with DNA: Put appropriate working solution of DNA and 0.5 mL of buffer solution into a 10 mL volumetric flask, vortex and then add 0.5 mL aloe-emodin solution. The mixture was diluted to the 5 mL scale mark by doubly distilled water and mixed thoroughly. The fluorescence spectra of aloe-emodin and the mixture of DNA and aloe-emodin were measured, which have a 480 nm excitation wavelength and a scanning range of 495-700 nm. Both slits of the excitation and emission were 10 nm in width.

Resonance light scattering spectrum of aloe-emodin interacting with DNA: Put 0.5mL working solution of aloe-emodin and 0.5 mL of buffer solution into a 10 mL volumetric flask,vortex and then add appropriate DNA solution The mixture was diluted to 5 mL scale mark by using doubly distilled water and mixed thoroughly. The resonance light-scattering spectrum was obtained by scanning simultaneously the excitation and emission monochromators of the RF-5301PC spectrofluorometer from 220 to 700 nm. The extent of light-scattering was measured at the maximum wavelength with slit width at 15.0 nm for the excitation and emission. The resonance light-scattering spectrum of 1.00×10^{-4} mol L^{-1} ethidium bromide interacting with DNA under the same condition was compared with the resonance light scattering spectrum of aloe-emodin interacting with DNA.

RESULTS AND DISCUSSION

Fluorescence spectrum of aloe-emodin interacting with DNA: Aloe-emodin which has intrinsic fluorescence emission is a typical planar polycyclic aromatic molecule. A series of assay solutions containing various concentrations of DNA and a constant concentration of aloe-emodin were prepared and the fluorescence measurements were carried out under the selected experiment conditions. The results are shown in Fig. 2. It is shown that DNA has fluorescence quenching effect to aloe-emodin. With the increase of the concentration of DNA gradually, the fluorescence peak intensity of aloe-emodin at 547 nm decreases correspondingly. Fluorescence occurs after material absorbing radiation, which intensity is related to the energy emitted by molecular transitions in decay of radiation. A number of small molecules can be combined with biological macromolecules and their spectral characteristics of energy transfer, causing the fluorescence quenching phenomenon. The phenomenon of DNA causing intrinsic fluorescence quenching of aloe-emodin indicates that some interaction takes place between aloe-emodin and DNA. Aloe-emodin may be intercalate with the DNA molecule and is wrapped up into a stable, non-luminous ground state material. aloe-emodin itself has an energy shift, which results in the weakening of the fluorescence intensity.

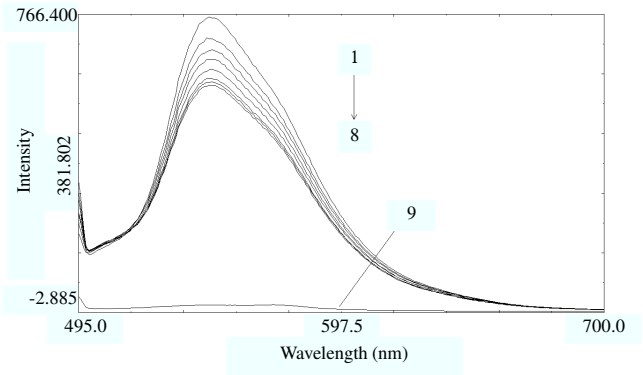

Fig. 2. Fluorescence spectra of aloe-emodin at different DNA concentration; 1-8: $C_{AE} = 1.00 \times 10^{-5}$ mol L^{-1}, C_{DNA} from 1 to 8 : 0, 0.378, 0.756, 1.134, 1.512, 1.890, 2.268, 2.646×10^{-5} mol L^{-1}, 9: $C_{DNA} = 2.646 \times 10^{-5}$ mol L^{-1}

Binding site and binding constants of aloe-emodin interacting with DNA: There may be two reasons of aloe-emodin fluorescence quenching: dynamic quenching and static quenching[8]. Dynamic quenching is the quenching caused by excited state aloe-emodin molecules colliding with the DNA molecules. It is the process associated with spontaneous emission and thereby shortening the life of excited states molecular, which has nothing to do with the structural changes of DNA. However, the static quenching is due to the non-luminous ground state compounds formed by aloe-emodin and DNA molecules. Dynamic quenching follows the Stem-Volmer equation[9]:

$$F_0/F = 1 + K_s[Q] = 1 + K_q\tau_0[Q] \quad (1)$$

where F_0 is the fluorescence intensity with the absence of quencher, F is the fluorescence intensity when adding the quencher, K_s is Stern-Volmer constant, [Q] is the concentration of quencher, K_q is the quenching constant, τ_0 is the average life expectancy of fluorescent molecules with the absence of quencher. Assuming this procedure as a dynamic quenching process, then according to eqn. 1, set aloe-emodin molecule fluorescence changes in F_0/ F as ordinate and the DNA concentration [Q] as abscissa to make a graph. Then the linear slope of the graph is the fluorescence quenching constant K_s. From

Fig. 3, it is observed that the quenching constant is 8.32×10^3 L mol^{-1}. However, the maximum quenching constant K of biological macromolecules dynamic quenching process[10] is smaller than 100 L mol^{-1}, which is far exceeded by the experiment value. Therefore it is deduced that the fluorescence quenching of aloe-emodin belongs to static quenching. For static quenching, the relationship between the fluorescence intensity and the quencher could be presented by eqn. 2[11]:

$$\lg[(F_0\text{-}F)/F] = \lg K_A + n\lg[Q] \quad (2)$$

Draw the plot between lg $[(F_0\text{-}F)/F]$ and lg[Q]. The linear slope is the binding sites n and the intercept is binding constants K_A. From Fig. 2, get the peak fluorescence at 547 nm and plot the curve shown by Fig. 4, which has the equation:

$$\lg[(F_0\text{-}F)/F] = 2.7608 + 0.7127 \lg[Q]$$

The linear correlation coefficient is 0.994, from the intercept and slope of the straight line we can get the binding site $n_{25\,^\circ C}$ of aloe-emodin interacting with DNA 0.7127 and the binding constant 5.765×10^2 L mol^{-1}.

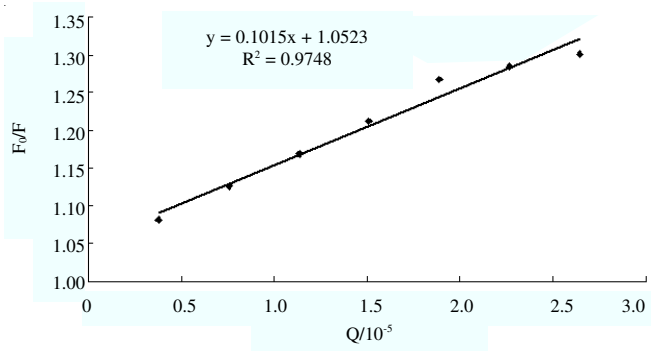

Fig. 3. Stern-Volmer curve of aloe-emodin quenched by DNA

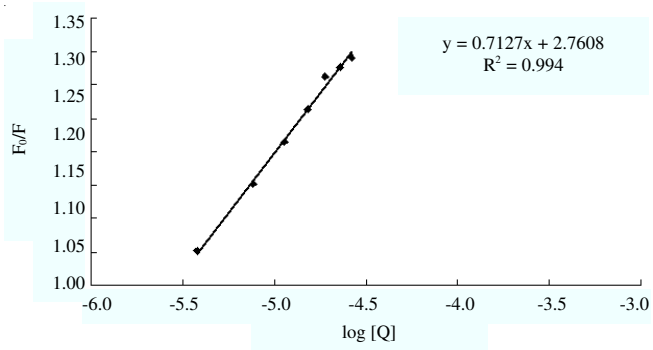

Fig. 4. Lineweaver-Burk curve of aloe-emodin quenched by DNA

Resonance light scattering spectrum of aloe-emodin interacting with DNA: The interaction procedure of aloe-emodin with fs-DNA in pH 7.4 BR was characterized by the resonance light scattering spectrum (Fig. 5). It is shown that the RLS signal of DNA itself is weak and aloe-emodin has four absorption bands centered at 240, 308, 468 and 557 nm, respectively. Present study selected the resonance scattering peak at 557 nm to investigate. Fix the concentration of aloe-emodin, the resonance scattering signal intensity gradually enhances with the DNA concentration's increase. We made a comparison between aloe-emodin and ethidium bromide (EB) at the same condition and found that ethidium bromide with the same concentration induces a larger resonance scattering

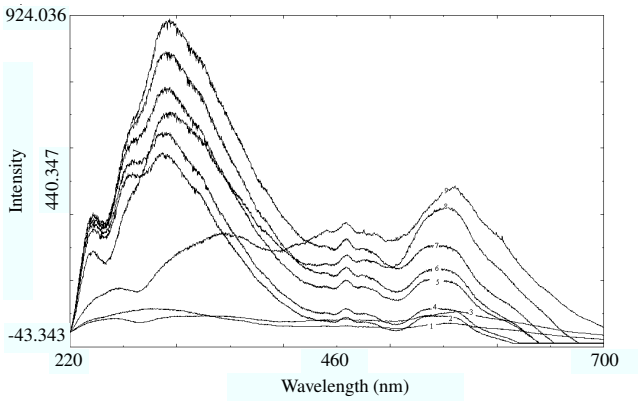

Fig. 5. Resonance light scatting spectra of DNA and aloe-emodin; 1: C_{DNA} = 3.78×10^{-6} mol L^{-1}, 2: C_{AE} = 1.00×10^{-5} mol L^{-1}, 3: C_{EB} = 1.00×10^{-5} mol L^{-1}, 4-8: C_{AE} = 1.00×10^{-5} mol L^{-1}, C_{DNA} from 4 to 8: 0.378×10^{-6}, 0.756×10^{-6}, 3.78×10^{-6}, 5.67×10^{-6}, 7.56×10^{-6} mol L^{-1}, respectively 9: C_{EB} = 1.00×10^{-5} mol L^{-1}, C_{DNA} = 3.78×10^{-6} mol L^{-1}

signal enhancement of DNA. It can be seen that the intensity of resonance scattering signal produced by 500 μL ethidium bromide interacting with DNA is close to that of 750 μL aloe-emodin and DNA. Resonance scattering results of this study show that the signal of aloe-emodin itself resonance scattering enhances with the DNA's accession in the selected buffer solution system. This resonance light scattering enhancing phenomenon shows that aloe-emodin may combine with DNA to form a compound, resulting in the formation of super-helical structure of nucleic acid. Resonance light scattering enhancing phenomenon is caused by delocalization of the electron excitation in this super-helix structure of nucleic acids. On the mechanism research of ethidium bromide interacting with DNA at present, ethidium bromide is recognized as a kind of DNA intercalator and can parallel intercalate into the base pairs of double-helical DNA[12,13]. Considering the strong enhanced RLS signals of aloe-emodin interacting with DNA was similar to that of ethidium bromide, we suspected that aloe-emodin is possible to intercalate into the base pairs of double-helical DNA.

Resonance light scattering spectrum of aloe-emodin under different solution conditions

pH value on the resonance light-scattering of aloe-emodin-DNA system: As seen from Fig. 6, different pH value will influence the resonance light scattering phenomenon of aloe-emodin-DNA system. In aloe-emodin-DNA system, the phenomenon of resonance light scattering strengthens in the acidic conditions, while under alkaline conditions, it barely exists. The structure of DNA under different pH values will change. The aloe-emodin's ionic state will also be influenced by the acidity and basicity of solution. Through the experiment it is obtained that the DNA structure is more easily to combine with aloe-emodin in the acidic condition. This phenomenon shows that the changing of pH value in environment influences the aloe-emodin and DNA binding capacity.

Amino acids on the resonance light-scattering of aloe-emodin-DNA system: Take C_{AE} = 1.00×10^{-5} mol L^{-1}, C_{DNA} = 3.78×10^{-6} mol L^{-1} mixed solution's resonance light scattering intensity value as the standard to study the effect of a variety of amino acids acting on aloe-emodin-DNA system. Record the relative increase (decrease) in percentage with + (-) note,

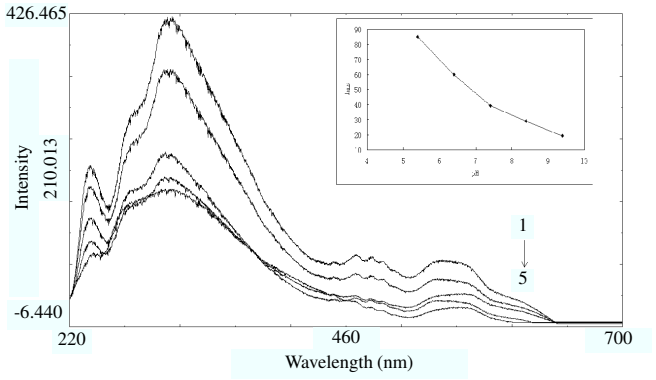

Fig. 6. Resonance light scatting spectra of DNA and aloe-emodin on different pH value; C_{AE} =1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; pH value from 1 to 5: 5.4, 6.4, 7.4, 8.4, 9.4, respectively, the spectral bandwidths of the excitation and emission being kept at 5.0 nm

respectively. "+" notes positive and "-" notes negative. The results from Figs. 7-14, Table-1 can be seen that among the investigated amino acids, the polar amino acids can increase the phenomenon of resonance light scattering in aloe-emodin-DNA system. Most of the non-polar amino acids would weaken the phenomenon of resonance light scattering, but tryptophan and valine would slightly increase it. From this it is observed that the polarity of environmental substances could influence the combination of aloe-emodin with DNA. In the investigation of amino acids influence on the resonance light scattering of aloe-emodin-DNA system, it is noted that the influence of histidine, phenylalanine, proline and tyrosine on the resonance scattering degree was greater than that of isoleucine and aspartate. Considering its structural characteristics, we can deduce that most of the amino acids which have large influence on the resonance scattering of the system contain the benzene ring or a heterocyclic structure. The structure containing a benzene ring or heterocyclic ring is similar with anthraquinone molecules. These molecules have π-π interaction force which makes it easier to interact with the DNA base pairs and hence competitively inhibit or enhance the combination of aloe-emodin and DNA's. From this it is noted that the structure of the material of surrounding environment will also influence the aloe-emodin and DNA binding capacity.

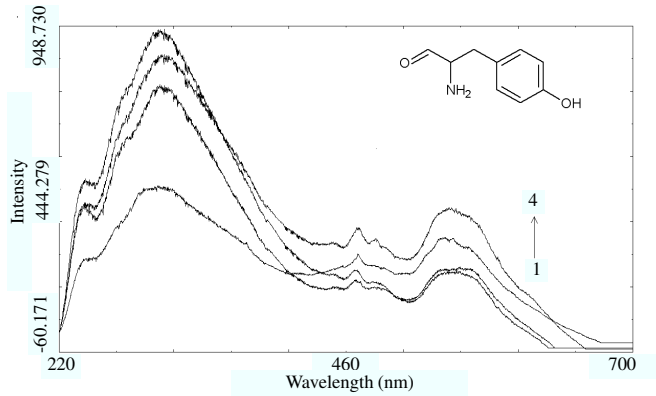

Fig. 7. Resonance light scatting spectra of DNA and aloe-emodin on different tyrosine concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{Tyr} from 1 to 4: 0, 1 × 10^{-6}, 4 × 10^{-6}, 1 × 10^{-5} mol L^{-1}, respectively

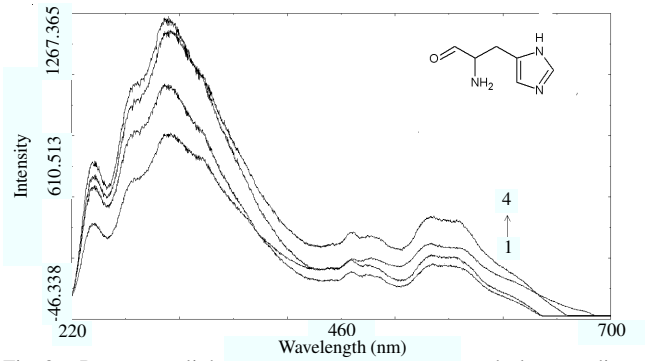

Fig. 8. Resonance light scatting spectra of DNA and aloe-emodin on different histidine concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{His} from 1 to 4: 0, 1 × 10^{-6}, 4 × 10^{-6}, 1 × 10^{-5} mol L^{-1}, respectively

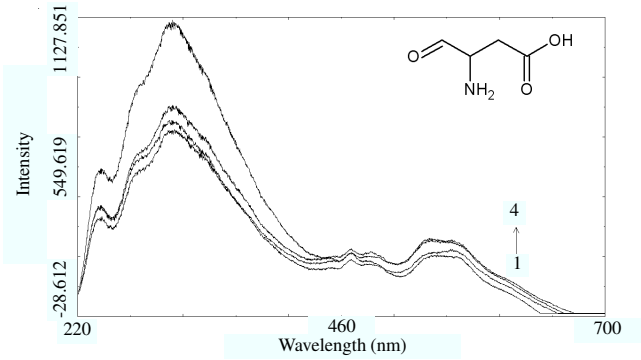

Fig. 9. Resonance light scatting spectra of DNA and aloe-emodin on different asparate concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{Asp} from 1 to 4: 0, 1 × 10^{-6}, 4 × 10^{-6}, 1 × 10^{-5} mol L^{-1}, respectively

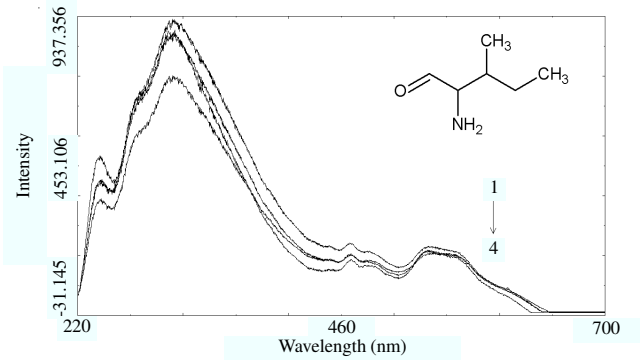

Fig. 10. Resonance light scatting spectra of DNA and aloe-emodin on different isolencine concentration; C_{AE} =1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{Ile} from 1 to 4: 0, 1 × 10^{-6}, 4 × 10^{-6}, 1 × 10^{-5} mol L^{-1}, respectively

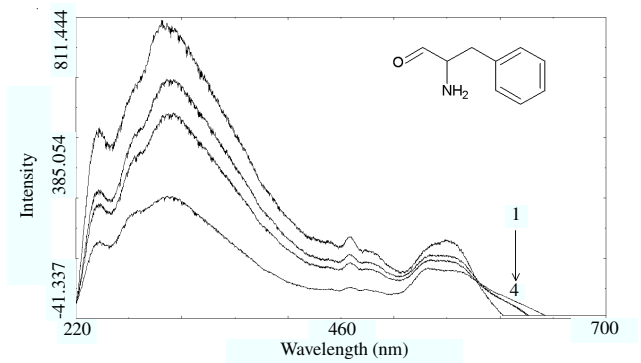

Fig. 11. Resonance light scatting spectra of DNA and aloe-emodin on different phenyl alanine concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{Phe} from 1 to 4: 0, 1 × 10^{-6}, 4 × 10^{-6}, 1 × 10^{-5} mol L^{-1}, respectively

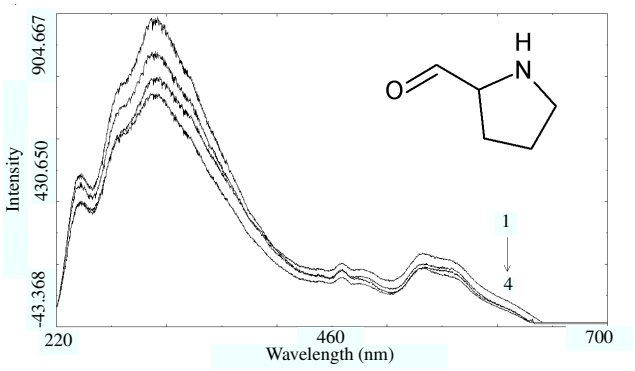

Fig. 12. Resonance light scatting spectra of DNA and aloe-emodin on different proline concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{Pro} from 1 to 4: 0, 1 × 10^{-6}, 4 × 10^{-6}, 1 × 10^{-5} mol L^{-1}, respectively

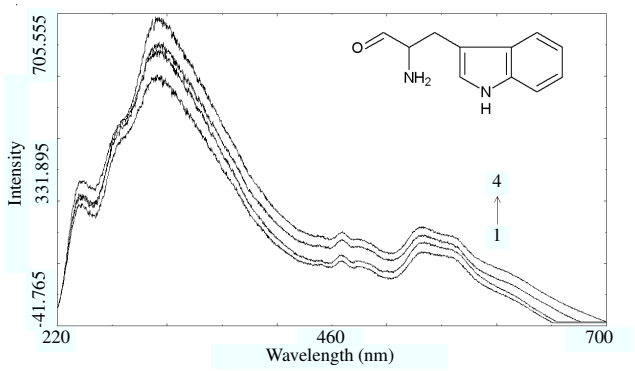

Fig. 13. Resonance light scatting spectra of DNA and aloe-emodin on different tryptophane concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{Trp} from 1 to 4: 0, 1 × 10^{-6}, 4 × 10^{-6}, 1 × 10^{-5} mol L^{-1}, respectively

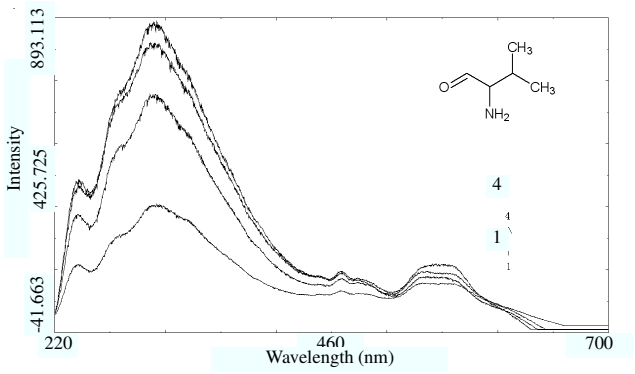

Fig. 14. Resonance light scatting spectra of DNA and aloe-emodin on different valine concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{Val} from 1 to 4: 0, 1 × 10^{-6}, 4 × 10^{-6}, 1 × 10^{-5} mol L^{-1}, respectively

TABLE-1
AMINO ACIDS' INFLUENCE ON RESONANCE LIGHT SCATTING SPECTRA OF DNA AND ALOE-EMODIN

	1 × 10^{-6} mol/L	4 × 10^{-6} mol/L	1 × 10^{-5} mol/L
Tyr	+7.4 %	+106.0 %	+135.0 %
His	+24.0 %	+59.0 %	+150.0 %
Asp	+11.5 %	+34.8 %	+42.0 %
Ile	–10.3 %	-10.3 %	-10.3 %
Phe	-32.0 %	-47.7 %	-54.5 %
Pro	-26.0 %	-33.0 %	-34.0 %
Trp	+9.1 %	+23.2 %	+37.3 %
Val	+12.1 %	+26.8 %	+48.1 %

Sugar, salt on the resonance light-scattering of aloe-emodin-DNA system: From Figs. 15 and 16, Table-2 it can be seen that sugar, salt and other environmental factors will also influence the aloe-emodin-DNA system. Sodium chloride has relatively larger effect on the resonance light scattering of aloe-emodin-DNA system. This may be because of the adding salt solution leads to the DNA molecules shrinking to form the super-helical structure. Since the body's fluid environment is mostly made up of sugar, salt and other materials, it is observed that the different body fluids concentrations will influence aloe-emodin and DNA's combination.

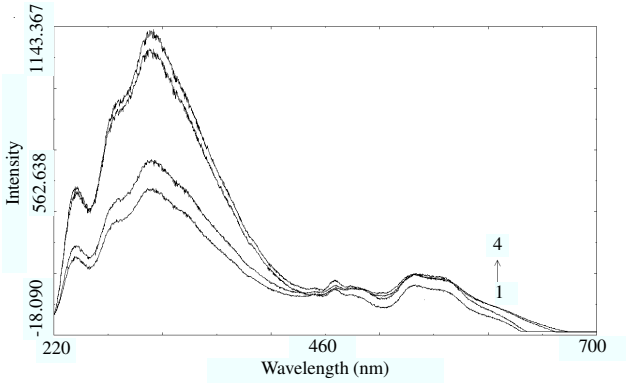

Fig. 15. Resonance light scatting spectra of DNA and aloe-emodin on different glucose concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; CDNA = 3.78 × 10^{-6} mol L^{-1}; $C_{Glucose}$ from 1 to 4: 0, 1 × 10^{-4}, 4 × 10^{-4}, 1 × 10^{-3} mol/L, respectively

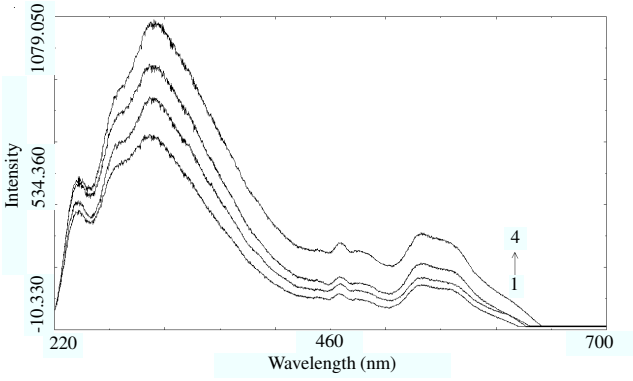

Fig. 16. Resonance light scatting spectra of DNA and aloe-emodin on different NaCl concentration; C_{AE} = 1.00 × 10^{-5} mol L^{-1}; C_{DNA} = 3.78 × 10^{-6} mol L^{-1}; C_{NaCl} from 1 to 4: 0, 1 × 10^{-2}, 4 × 10^{-2}, 1.0 × 10^{-1} mol/L, respectively

TABLE-2
GLUCOSE AND NaCl's INFLUENCE ON RESONANCE LIGHT SCATTING SPECTRA OF DNA AND ALOE-EMODIN

	1 × 10^{-2} mol/L	4 × 10^{-2} mol/L	1 × 10^{-1} mol/L
NaCl	+28.5 %	+85.7 %	+198 %
	1 × 10^{-4} mol/L	4 × 10^{-4} mol/L	1 × 10^{-3} mol/L
Glucose	+37.2 %	+37.3 %	+37.3 %

Conclusion

This study found that aloe-emodin can be intercalated with DNA molecules, with the binding constant 5.765 × 10^{2} L mol^{-1} and binding sites 0.7127. Amino acids, pH, sugar, salt and other environmental factors can influence the stability of DNA intercalate with aloe-emodin. Therefore, the changing environ-

mental factors can affect the interaction between aloe-emodin and DNA, so as to achieve the purposes of attenuation and synergistic effect. The obvious difference between Chinese and western medicine is that Chinese medicine is usually not a single medicinal composition. Even the using of a single Chinese herbal medicine, particularly, the single toxic Chinese herbal medicine is extremely rare. Actually Chinese medicine usually uses multiple herbs to compose prescriptions to achieve the compatibility attenuation and synergistic effect. Just because the ingredients of Chinese medicine are too complex to clearly analyze and the mutual interaction and restriction between the various components exists, the method simply making a copy of conventional research techniques to study the drug effects of traditional Chinese medicine and the potential toxicity mechanism becomes very difficult. Additionally, Chinese medicine has the attenuation and synergistic effect through compatibility method. But its theories and methods are still in a simple parampara stage so far, which means that it mostly depends on experience lacking of fundamental modern science and experimental evidence. Therefore, the spectroscopy result and method based on studying aloe-emodin interacting with DNA and the environment influence introduced in this studies could start a new research exemplification to study the drug effects of traditional Chinese medicine and the potential toxicity mechanism and then, to study the compatibility attenuation and synergistic effect of Chinese medicine and also the way to avoid the toxic and side effects.

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