

# CHEMISTRY & BIOLOGY INTERFACE

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## Spectroscopic and Molecular docking studies on the interaction of an anticancer $\beta$ -Carboline Alkaloid, Harmine with GC and AT specific DNA oligonucleotides

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**Abstract:** In the present study the binding characteristics of Harmine with various DNA oligonucleotides have been studied by UV-Vis, fluorescence spectroscopy and molecular docking methods. UV absorption studies, Fluorescence quenching and Iodide quenching experiments confirmed intercalation type of binding of Harmine with short sequence specific DNA oligonucleotides. Binding constants of Harmine with sequence specific DNA oligomers were calculated by Fluorescence and absorption spectroscopic methods. For GC rich sequences binding constants were found in the order of  $10^5 \text{ M}^{-1}$  while for AT rich sequences it the values of binding constants were in the order of  $10^3 \text{ M}^{-1}$ , which clearly indicated that Harmine showed greater interaction with GC rich sequences compared to AT rich sequences. The temperature effect studies and pH studies further supported the intercalative mode of binding. Molecular docking studies and the calculated thermodynamic parameters, Gibbs free energy ( $\Delta G$ ), Enthalpy change ( $\Delta H$ ) and Entropy change ( $\Delta S$ ) studied at different temperature indicated that the hydrogen bonds, van der Waals interactions and hydrophobic interactions have a major role in the recognition of Harmine to the proposed DNA oligomers.

**Keywords:** Harmine, Intercalation, Fluorescence, Drug-DNA binding, GC base specificity, Molecular Docking

### 1. Introduction

The biological activities of many anticancer or antitumor drugs may be often explained in terms of their interactions with nucleic acids (DNA or RNA). These studies help in designing and synthesizing more potent antitumor drugs which target nucleic acids in a sequence

specific manner. A bulk of research in this area to achieve the target has been carried out and researchers are still looking for more competent drug molecules. Several analytical techniques like UV-Vis absorption, fluorescence, and CD spectroscopic methods; ITC, NMR, cyclic voltammetry etc. have been employed in the interpretation of interactions of different

anticancer drugs with nucleic acids.

Harmin (Fig. 1), a  $\beta$ -Carboline alkaloid, isolated from the seeds of *Peganum harmala* (Syrian Rue, family Zygophyllaceae), recently has drawn attention due to its antitumor activities. Harmin (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole) is a tricyclic  $\beta$ -carboline alkaloid. It is widely distributed in nature. Common sources of Harmin are various plants, marine creatures, insects, mammals, human tissues and body fluids. Harmin possess antimicrobial, antiplasmodial, antifungal, antioxidative, antitumor, antimutagenic, cytotoxic and hallucinogenic properties [1-8]. It interacts with DNA via intercalative modes and cause major DNA structural changes. Different techniques have been used for the study of interaction of Harmin with natural DNA like CT-DNA, superhelical plasmid pBR322 DNA, Salmon fish sperm DNA etc. [9-13]. Nevertheless interactions of Harmin with short sequence specific oligonucleotides have not been studied yet. Although, various studies reported the intercalation mode of binding in the Harmin-DNA complex, yet a little is known about the sequence specificity of bases in DNA structure, at the position of binding of Harmin. Therefore, a detailed study on the DNA base sequence specificity of binding of Harmin was needed to be furnished. In order to verify the specificity we have studied the interaction of Harmin with sequence specific DNA oligomers with the help of absorption and fluorescence spectroscopy techniques. To complement the structural data on the Harmin-DNA interaction, it is necessary to gain an insight into the stability and binding energies of complexation. The present study also discusses the approach required for the elucidation of the thermodynamic aspects of binding. The structural model of Harmin-DNA complex was also obtained by molecular docking studies to find structural details of the complex.

The study of mode of complexation of Harmin with DNA was carried out using four DNA oligomers. These four selected oligomers have sequence specific center cores.

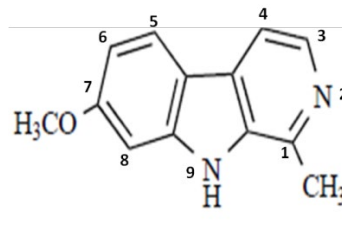


**Fig. 1:** Harmin

The sequence specific core of these oligomers is helpful in determining the AT/GC specificity of Harmin. Four DNA oligomers proposed for the study are as followed:

1. DNA-1: 5'-d(GATGGCCATC)<sub>2</sub>
2. DNA-2: 5'-d(GATCCGGATC)<sub>2</sub>
3. DNA-3: 5'-d(GGCAATTGCC)<sub>2</sub>
4. DNA-4: 5'-d(GGCTTAAGCC)<sub>2</sub>

## 2. Materials & Methods

### 2.1 Chemicals & Reagents

Proposed DNA sequences, Harmin, other chemicals like disodium hydrogen phosphate, sodium dihydrogen phosphate, used for the buffer preparation and potassium iodide (KI) were purchased from Sigma-Aldrich Chemical Co., New Delhi, India. Harmin and DNA sequences were used without further purification. DNA samples were prepared in 10 mM sodium phosphate buffer at pH 7.6. ds-DNAs were obtained by regular renaturation experiments [14]. The concentration of DNA sequences were determined spectrophotometrically using the molar extinction coefficients,  $\epsilon_{260} = 95,000$  for DNA-1,  $\epsilon_{260} = 96,600$  for DNA-2,  $\epsilon_{260} = 92,600$  for DNA-3 and  $\epsilon_{260} = 93,200$  for DNA-4. Stock solution of Harmin was prepared by dissolving Harmin in ethanol with gentle warming. Concentration of Harmin

was  $1.0 \times 10^{-3}$  Mol L<sup>-1</sup> and it was determined spectrophotometrically. All the solutions were freshly prepared and stored below freezing point. All experiments were conducted at 25 °C in 10 mM sodium phosphate buffer, pH 7.6. Deionized and doubled distilled water and analytical grade reagents were used in all the experiments.

## 2.2 Absorption Studies

The absorption spectra of Harmine mixed with or without DNA were obtained using Agilent CARY60 UV-Vis spectrophotometer equipped with quartz cuvette having 1 cm optical path length. The spectrophotometric titrations were performed by keeping the concentration of Harmine constant while varying the DNA concentration as described earlier [15-16].

## 2.3 Fluorescence Titrations & Iodide Quenching Studies

Fluorescence spectra were recorded on Varian Cary Eclipse Fluorescence Spectrophotometer equipped with a xenon lamp and a thermostat bath using fluorescence free quartz cuvette of 1 cm path length. The excitation and emission band pass were 5 nm each. The excitation wavelength for Harmine was 320 nm and Harmine showed its emission maxima at 420 nm. Fluorescence quenching titrations were performed by keeping the concentration of Harmine constant and increasing the concentration of DNA decamer as previously reported [17-18]. This method was repeated for all four DNA oligomers separately. The effect of pH on the binding affinity of Harmine with the proposed DNA oligomers was studied by conducting the fluorescence titrations in phosphate buffer of pH 6.0, 7.6 and 8.0. Binding constants were calculated at different pH for the Harmine-DNA complexes with all four proposed DNA oligomers (DNA-1 to DNA-4).

Quenching studies were carried out using the anionic quencher KI at a constant molar ratio of nucleic acid/alkaloid (P/D) by monitoring the fluorescence intensity changes at 420 nm as a function of the quencher concentration. The data were plotted as  $F_0/F$  versus quencher concentration  $[Q]$  according to the Stern–Volmer equation, as described earlier [15].

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

where  $F_0$  and  $F$  are the fluorescence intensities of the drug complex in the absence and in the presence of the quencher and  $K_{sv}$  is the Stern–Volmer quenching constant, which is a measure of the efficiency of the quencher.

## 2.4 Evaluation of Binding Constants

Quantitative measurements of the binding of Harmine with DNA oligomers (DNA-1 to DNA-4) were obtained from the absorbance and fluorescence spectroscopic studies. To evaluate the non-specific ligand interactions with DNA oligomers as reported earlier, the double reciprocal method was employed [14, 17]. The DNA-binding constants were calculated using double reciprocal method (plot of  $1/[DNA]$  vs  $1/[A_0 - A]$  or  $1/[E_0 - E]$ , where  $A_0$  is the initial absorbance of drug,  $A$  is the absorbance in presence of DNA.  $E_0$  is the emission value of drug;  $E$  is the emission value in the presence of DNA). Straight line with a positive slope was obtained in each case and DNA binding constant for each complex was calculated by taking ratio of intercept to slope.

## 2.5 Effect of Temperature & Evaluation of Thermodynamic Parameters

Effect of temperature on binding of Harmine with all four DNA oligomers was monitored by carrying out fluorescence titration with fixed drug concentration and increasing DNA concentration at 15, 20, 30 and 40 °C.

Thermodynamic parameters were estimated by the analysis of  $\ln K$  vs.  $1/T$  plot (Van't Hoff plot) obtained by the experimental data at above temperatures [19]. The gradient of this straight line of  $\ln K$  versus  $1/T$  is equal to  $-\Delta H/R$  which indicates the value of  $\Delta H$ .  $\Delta G$  and  $\Delta S$  can be calculated from the following fundamental thermodynamic relationships:

$$\ln K = - \frac{\Delta H}{RT} \quad (2)$$

$$\Delta G = - RT \ln K \quad (3)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (4)$$

### 2.6 Molecular Docking Studies

All the docking experiments were performed using the AutoDock 4.2 program package from the Scripps Research Institute [20]. For docking experiments the PDB files of DNA were obtained from protein data bank (PDB ID: 4BZV, 1G3X). The intercalated ligands in the PDB files were removed using UCSF Chimera program to develop an intercalation cavity in both the PDB files. The obtained DNA structures, having an empty intercalation cavity were energy minimized by XPLOR program. As the Autodock program uses PDBQT files for docking, the PDB files of ligand and modified DNA structures were converted into PDBQT file format using MGLTools (version 1.5.4) [21]. For docking experiments a 3-dimensional grid box of  $54 \times 60 \times 54$  with grid spacing  $0.442 \text{ \AA}$  was prepared for both the DNA structures and grid box center was at  $x = 32.36$ ,  $y = 31.09$ ,  $z = 31.32 \text{ \AA}$ . All other parameters were kept as default. The search parameter used for docking was Lamarckian genetic algorithm. 10 different poses were recorded for each complex. The analysis of docked structures was performed by Discovery Studio Visualizer from Accelrys Inc.

## 3. Results and Discussion

### 3.1 Absorption Spectroscopic Studies

In general, the hyperchromism and hypochromism are regarded as spectral features for the change in the DNA double-helix structure when DNA interacted with other molecules. The hyperchromism originates from the breakage of the DNA duplex secondary structure whereas the hypochromism originates from the stabilization of the DNA duplex, either by the intercalation binding mode or the electrostatic effect of small molecules [22]. Harmine exhibited peak maxima at  $247 \text{ nm}$  and  $320 \text{ nm}$ . The maxima at  $247 \text{ nm}$  was not found suitable for inspection of the interaction of Harmine with DNA in order to avoid overlapping of spectra of drug and DNA as DNA molecule also shows absorption at  $260 \text{ nm}$ . So absorption peak at  $320 \text{ nm}$  was marked for further study. In the present study the concentration of the drug was kept constant, while the concentration of DNA oligomers was increased by adding small aliquots of DNA solution. For each addition, the UV absorption spectrum was recorded. As shown in Fig. 2, at each addition of aliquot of DNA oligomers, the absorption peak of Harmine at  $320 \text{ nm}$  showed a decrease in the intensity with red shift. This effect was observed for all four DNA duplexes (DNA-1 to DNA-4) with varying sequence context. These results are same as for observed strong hypochromism and red shifts in the absorption spectra when small molecules intercalate into the base stack [23]. Therefore, these results indicated the intercalation mode of binding for Harmine DNA complexation. To further gain an insight into the base specificity of Harmine towards DNA, we used absorption data and calculated binding constants by plotting double reciprocal plot i.e.  $1/[DNA]$  vs  $[1/A_0 - A]$ . All complexes result into a straight line and binding constant was calculated by taking ratio of intercept to slope. Binding constants came out in the order of  $10^3$ - $10^5 \text{ Mole}^{-1}$ . Similar results have been reported for several drug molecules binding

to DNA structures [24]. Since all four DNA sequences contain equal numbers of GC base pairs (six) having different sequence specific central core in their structures as discussed before, it seems likely that the location of GC base pairs on the DNA molecule affects the binding of Harmine. Harmine showed strongest binding with DNA-1: 5'-d(GATGGCCATC)<sub>2</sub> compared to other oligomers DNA-2, DNA-3 and DNA-4. Therefore it was concluded after the close inspection of DNA binding constants (shown in Table 1) that Harmine showed a greater DNA binding specificity towards the 5'-GC-3' sequences.

**Table-1: Binding constants (K) per mole calculated by absorption studies**

| Complex       | Binding constant (K) Mole <sup>-1</sup> |
|---------------|-----------------------------------------|
| Harmine-DNA:1 | $1.9 \times 10^5$                       |
| Harmine-DNA:2 | $5.5 \times 10^4$                       |
| Harmine-DNA:3 | $1.2 \times 10^3$                       |
| Harmine-DNA:4 | $1.6 \times 10^4$                       |

### 3.2 Fluorescence Spectra

Absorption study results were further supported by fluorescence quenching study. To evaluate its interaction with DNA oligomers, Harmine was used as fluorescent probe as the fluorescence of DNA is very weak. Harmine shows its emission band at 420 nm when excited at 320 nm. Drug with a fixed concentration was taken in cuvette and spectra were obtained by adding aliquots of DNA samples as reported earlier [25-26]. These titrations were performed with all four DNA oligomers. With addition of each DNA aliquot, a decrease in the intensity of emission peak of Harmine at 420 nm was noticed and red shift was also observed on addition of each DNA aliquots as shown in Fig. 3. This result is in favor to the observed hypochromism and significant red shifts in the fluorescence maxima when drugs intercalate into the DNA helix [27]. These remarkable spectral changes revealed  $\pi$ - $\pi$ -stacking interactions between the chromophore of these molecules and DNA oligomers which is a driving force for intercalation. So it can be assumed that Harmine binds with DNA oligomers in intercalative mode. To support the results of absorption study, base specificity of Harmine towards DNA was determined

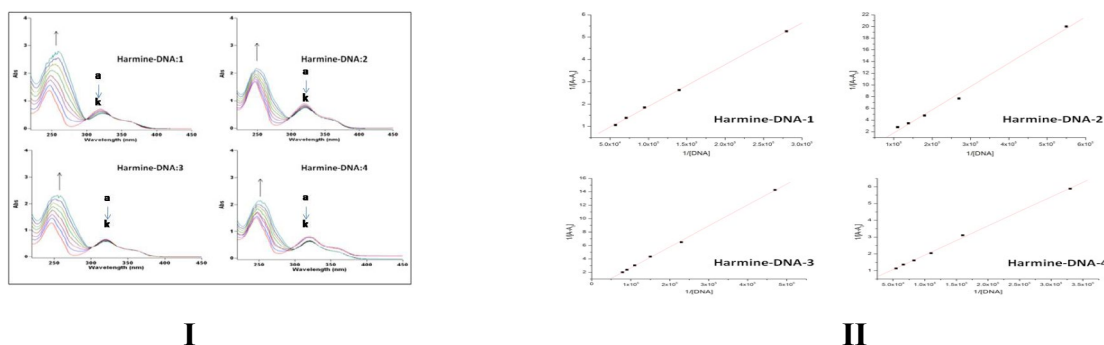


**Fig. 2: I) UV-Vis absorption spectra of a: Harmine ( $1.6 \times 10^{-5}$  Mol L<sup>-1</sup>) in the presence of increasing amount of i) b-k,  $2.0-20 \times 10^{-5}$  Mol L<sup>-1</sup> DNA:1 ii) b-k,  $1.2-12 \times 10^{-5}$  Mol L<sup>-1</sup> DNA:2 iii) b-k,  $3.5-35 \times 10^{-5}$  Mol L<sup>-1</sup> DNA:3 iv) b-k,  $3.6-36 \times 10^{-5}$  Mol L<sup>-1</sup> DNA:4 respectively from top to bottom (pH = 7.6, T = 298 K) II) Double Reciprocal Plot of Harmine-DNA:1, DNA:2, DNA:3 and DNA:4 complexes for evaluation of binding constant from absorption study**

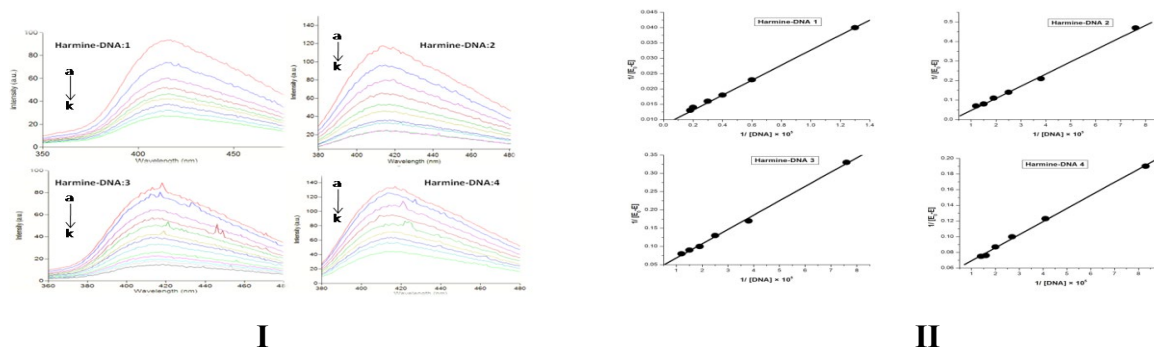


**Fig. 3: I) Fluorescence spectra of a: Harmine ( $1.6 \times 10^{-5}$  Mol L $^{-1}$ ) in the presence of increasing amount of i) b-k, 1.6-16  $\mu$ Mol L $^{-1}$  DNA:1 ii) b-k, 1.8-18  $\mu$ Mol L $^{-1}$  DNA:2 iii) b-k, 2.2-22  $\mu$ Mol L $^{-1}$  DNA:3 iv) b-k, 1.1-11  $\mu$ Mol L $^{-1}$  DNA:4 respectively from top to bottom ( pH = 7.6, T = 298 K,  $\lambda_{ex}$  = 320 nm) II) Double reciprocal plot of Harmine-DNA:1, DNA:2, DNA:3 and DNA:4 complexes for evaluation of Binding constants from fluorescence study**

by calculating the binding constants for each Harmine-DNA complex. A straight line was obtained in case of all complexes and binding constants were calculated by ratio of intercept to slope as described above. DNA Binding constants were found to be comparable with the binding constants calculated by UV-absorption method. The binding constants calculated from absorption and fluorescence quenching studies are in good agreement with each other. Comparison of the same results obtained in the absorption spectroscopic method and the fluorescence quenching methods indicated the intercalative mode of complex formation between Harmine and DNA oligomers. The greatest value of binding constant for Harmine-DNA: 1 complex like as observed in the absorption study, indicated the highest binding affinity of Harmine towards DNA: 1, having GC specific central core and base specificity of Harmine towards 5'G-C 3' sequences (Table 2).

Table 3 summarizes the effect of pH on DNA binding of Harmine with proposed DNA sequences (DNA-1 to DNA-4). At a glance, it was noticed that binding constant increased slightly in acidic medium while in basic medium slight decrease in the value of K was observed. No marked variation was exhibited in all cases.

So it was inferred that the pH of medium did not affect the binding of Harmine with DNA sequences distinctly.

**Table-2: Binding constants (K) per mole calculated by fluorescence studies**

| Complex       | Binding constant (K) Mole $^{-1}$ |
|---------------|-----------------------------------|
| Harmine-DNA:1 | $4.4 \times 10^5$                 |
| Harmine-DNA:2 | $5.5 \times 10^4$                 |
| Harmine-DNA:3 | $4.5 \times 10^3$                 |
| Harmine-DNA:4 | $4.6 \times 10^4$                 |

### 3.3 Iodide Quenching Studies and Elucidation of the Mode of Binding

In order to decide the mode of binding of the Harmine-DNA complexation, the fluorescence quenching method was used. KI was used as quencher. This study helped us to examine the degree of accessibility of drug molecule to the KI quencher and the steric bulk property of the DNA. A highly negatively charged quencher was supposed to experience the repulsive force by the negatively charged phosphate backbone

of DNA. Therefore, the drug intercalated between the base pair stacks, would be protected from the iodide ions and this would also result in a decrease in the value of Stern-Volmer constant [28]. On the other hand, in the case of minor groove binders, drugs should be quenched readily by iodide quenchers therefore a little difference in Stern-Volmer constant should be observed [29, 30]. Thus iodide anions were selected to determine the binding mode of Harmine with all four proposed DNA sequences. A low quenching of fluorescence of Harmine by iodide ions was observed in case of DNA:1 and DNA:2 and more quenching in case of DNA:3 and DNA:4, indicating that Harmine molecules were present in more protective environment with DNA:1 and DNA:2 as compared to DNA:3 and DNA:4. Stern-Volmer plots were obtained for each complex shown in Fig. 4. There was a great decrease in magnitude of  $K_{sv}$  in each case as shown in Table 4. On comparison of  $K_{sv}$  obtained for all the Harmine-DNA complexes, it can be inferred that Harmine was protected from solvent molecules indicating strong intercalative mode of binding with all DNA oligomers and it was also observed that there was a great decrease in  $K_{sv}$  in the case of the Harmine-DNA:1 complex which suggested the base specificity of Harmine towards GC base pairs.

**Table-3: Binding constants of Harmine-DNA complexes at pH 6.0, 7.6 and 8.0**

| Complex       | pH  | Binding Constant (K) |
|---------------|-----|----------------------|
| Harmine-DNA:1 | 6.0 | $3.0 \times 10^4$    |
|               | 7.6 | $4.4 \times 10^5$    |
|               | 8.0 | $1.2 \times 10^4$    |
| Harmine-DNA:2 | 6.0 | $5.7 \times 10^4$    |
|               | 7.6 | $5.5 \times 10^4$    |
|               | 8.0 | $2.7 \times 10^4$    |
| Harmine-DNA:3 | 6.0 | $2.2 \times 10^4$    |
|               | 7.6 | $4.5 \times 10^3$    |
|               | 8.0 | $6.1 \times 10^4$    |
| Harmine-DNA:4 | 6.0 | $8.8 \times 10^4$    |
|               | 7.6 | $4.6 \times 10^4$    |
|               | 8.0 | $6.8 \times 10^4$    |

**Table-4: Stern-Volmer constants ( $K_{sv}$ ) for Harmine-DNA complexes**

| Complex       | Stern-Volmer Constant ( $K_{sv}$ ) |
|---------------|------------------------------------|
| Harmine-KI    | 170                                |
| Harmine-DNA:1 | 80                                 |
| Harmine-DNA:2 | 107                                |
| Harmine-DNA:3 | 110                                |
| Harmine-DNA:4 | 140                                |

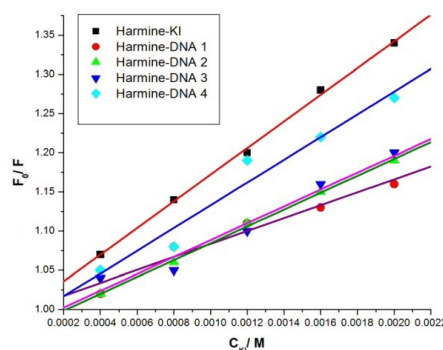


**Fig. 4: Stern–Volmer plots for quenching of Harmine by KI in the absence and presence of DNA oligomers**

### 3.4 Effect of Temperature and Calculation of Thermodynamic Parameters

In aiding rational drug design process, characterizing the energetics of the interaction is an essential component. Therefore energetics of binding of Harmine with proposed DNA oligomers was studied. In this work, the effect of temperature on DNA-Harmine complex fluorescence quenching was also examined. Table 5 summarizes the binding constants of Harmine with all four DNA oligomers at temperature 15, 20, 30, 40 °C. Results showed that binding constant decreased with increase in temperature in each case which indicated that quenching mechanism followed in the Harmine-DNA complex is by complex formation i.e.

static quenching rather than dynamic quenching [31].

Theoretically, the thermodynamic parameters, free energy, enthalpy and entropy changes which describe the binding of drug to DNA are determined by the following contributions: (i) the molecular interactions between the bound drug and the DNA-binding site such as H-bonding, hydrophobic interactions, van der Waals interactions and electrostatic interactions; (ii) conformational changes either in DNA or in the ligand molecule on complexation, (iii) contributions may come from coupled processes such as ion release, proton release or changes in hydration [32]. These thermodynamic parameters could be calculated by using Van't Hoff plots and eq. 2 and 3. Van't Hoff plots were obtained by plotting  $1/T$  vs  $\ln K$ . Fig. 5 shows Van't Hoff plots for temperature dependent Harmine-DNA complexation. The data fits to straight line for each DNA oligomers. The value of thermodynamic parameters evaluated from these plots for the Harmine-DNA oligomers are presented in Table 6.

The Van't Hoff plots show linear relationship and provide negative enthalpy changes in all cases analogous to that observed for other intercalators [33-35]. It can be concluded from Table 6 that for all cases, change in Gibb's free energy is negative indicating that complex formation is spontaneous. Association is driven by favorable  $\Delta H$ s (negative enthalpies) and therefore, is exothermic, while the entropy changes (negative entropies) are unfavorable. The large negative enthalpy of the binding observed here particularly in the Harmine-DNA: 1 and Harmine-DNA: 2 systems are generally typical for intercalative interaction of small molecules to nucleic acids [36]. Negative value of  $\Delta H$  and  $\Delta S$  in all cases suggested that non-bonded interaction like van der Waals and H-bonding played an important role in

each complex. This is also reported that minor groove binding is almost entirely driven by hydrophobic interactions, whereas intercalation is supported almost entirely by non-covalent molecular interactions and the hydrophobic effect [37]. So our results are in with good agreement with these facts. The unfavorable entropy with DNA oligomers may be attributed to the conformational change of DNA oligomers upon binding to Harmine [38, 39]. Table 6 showed that enthalpy changes were larger for Harmine-DNA:1 and Harmine-DNA:2 (GC specific central core) complexes as compared to other complexes. It suggested that the complex formed between Harmine and DNA:1 and DNA:2 are energetically more stable than formed with other DNA oligomers. Thus it can be concluded that Harmine binds preferably to GC specific sites.

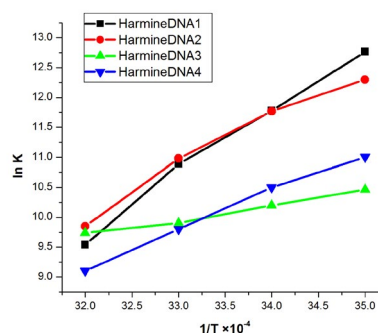


**Fig. 5: Van't Hoff Plots for Harmine with various DNA oligomers**

**Table-5: Binding constants of Harmine with DNA oligomers at different temperature**

| Complex       | Binding Constants (K) per mole |                   |                   |                    |
|---------------|--------------------------------|-------------------|-------------------|--------------------|
|               | 15° C                          | 20° C             | 30° C             | 40° C              |
| Harmine-DNA:1 | $22 \times 10^4$               | $13 \times 10^4$  | $6.3 \times 10^4$ | $5.23 \times 10^3$ |
| Harmine-DNA:2 | $35 \times 10^4$               | $11 \times 10^4$  | $8.9 \times 10^4$ | $1.4 \times 10^4$  |
| Harmine-DNA:3 | $3.5 \times 10^4$              | $2.4 \times 10^4$ | $2.0 \times 10^4$ | $1.7 \times 10^4$  |
| Harmine-DNA:4 | $6.0 \times 10^4$              | $4.0 \times 10^4$ | $1.2 \times 10^4$ | $0.9 \times 10^4$  |

**Table-6: Thermodynamic parameters  $\Delta H$ ,  $\Delta G$  and  $\Delta S$  for Harmine complexed with different DNA oligomers**

| Complex       | $\Delta H$<br>(kcal mol <sup>-1</sup> ) | $\Delta G$ (kcal mol <sup>-1</sup> ) |       |       |       | $\Delta S$ (cal mol <sup>-1</sup> ) |       |       |       |
|---------------|-----------------------------------------|--------------------------------------|-------|-------|-------|-------------------------------------|-------|-------|-------|
|               |                                         | 15°                                  | 20°   | 30°   | 40°   | 15°                                 | 20°   | 30°   | 40°   |
| Harmine-DNA:1 | -19.5                                   | -5.9                                 | -6.7  | -6.8  | -5.9  | -47.2                               | -43.6 | -41.9 | -43.4 |
| Harmine-DNA:2 | -23.8                                   | -7.0                                 | -6.8  | -6.6  | -5.2  | -58.3                               | -58.0 | -56.7 | -59.4 |
| Harmine-DNA:3 | -2.7                                    | -5.7                                 | -5.84 | -5.83 | -6.03 | 10.0                                | 10.5  | 10.2  | -10.5 |
| Harmine-DNA:4 | -12.6                                   | -6.2                                 | -5.9  | -5.7  | -5.6  | -22.2                               | -22.8 | -22.7 | -22.3 |

### 3.5 Molecular Docking Studies

Molecular docking technique is a new and important technique to generate the structural scaffold of the drug-DNA complex and also a mechanistic technique in which a drug molecule is placed in between the DNA base pairs mainly in a non-covalent fashion [40]. Molecular modeling technique allows the flexibility within the ligand to be modeled and can utilize more detailed molecular mechanics to calculate the

energy of the ligand in the context of the putative active site [41-42]. In our study, we had turned ligand flexible and kept the DNA sequence rigid in order to attain different binding mode of ligand with DNA sequences. Among the 20 runs performed in each docking, the ten most favorable runs were analyzed. Results indicated that Harmine intercalated in the cavities of both DNA structures having GC and AT specific intercalation cavities (Fig. 6). This conclusion was consistent with the results of experimental methods and showed the power of theoretical calculations in predicting the site of interaction on DNA for a small molecule. The planer ring structure Harmine fits in the cavity of GC and AT base pairs in the DNA oligomer. This conclusion confirmed the intercalation mode of binding of Harmine with DNA. Preferential binding of Harmine leads to van der Waals, electrostatic and hydrophobic interactions with DNA functional groups same as the results of thermodynamic calculations. From the table 7, on comparison of binding energy and binding constants, it is clear that experimental and theoretical values of binding energy and binding constants are comparable. It is also notified that Harmine binds preferentially with G-C base

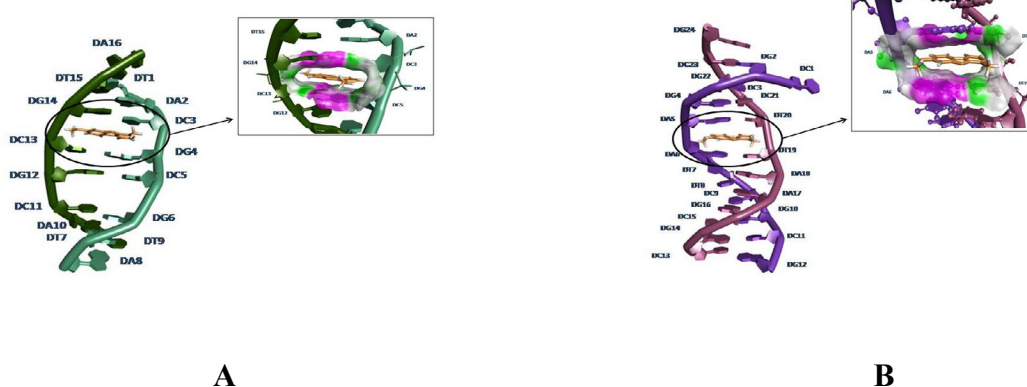


**Fig. 6: A) Harmine binding with intercalation cavity at 5'-Gp.C-3' of duplex DNA, inset showing the zoomed view of Harmine sandwiched between GC base pairs (PDB ID: 4BZV) B) Harmine binding with intercalation cavity at 5'-Ap.A-3' of duplex DNA (PDB ID: 1G3X) inset showing the zoom view of Harmine sandwiched between AA base pairs.**

pairs with lesser amount of binding energy. It contributes as the evidence for the G-C specific binding of intercalation mode [43]. It is clearly notified from the results that H-bonding between N1 atom of Harmine and phosphate backbone of DNA was also involved in the formation of the complex of Harmine with DNA (Fig. 6).

**Table-7: Binding energy and Binding constants for Harmine-DNA complexes calculated from docking method**

| Complex          | Binding Energy ( $\Delta G_{\text{pred}}$ ) | Binding Constant ( $K_{\text{pred}}$ ) |
|------------------|---------------------------------------------|----------------------------------------|
| Harmine-PDB 4BZV | -6.6                                        | $20 \times 10^4$                       |
| Harmine-PDB 1G3X | -5.9                                        | $5.0 \times 10^4$                      |

## Conclusions

We have studied the mode of interaction between Harmine and base specific DNA oligomers by spectroscopic methods and molecular docking technique. Absorption maxima of Harmine showed hypochromic effect on addition of DNA sequences suggesting intercalation of Harmine in proposed DNA oligomers. Fluorescence intensity of Harmine decreased upon binding to all four DNA sequences confirming DNA binding of Harmine. Binding constants showed that Harmine prefers to bind GC specific sites as compared to AT specific sites. The Extent of decrease in fluorescence intensity is larger at lower temperature suggesting that all four complexes followed static quenching mechanism. All the Harmine-DNA complexes produced the positive slope in the Van't Hoff plot. These observations suggest that binding of Harmine with four DNA sequences was energetically favorable. Thermodynamic parameters and molecular docking studies suggested that the forces involved in the

complexation of Harmine with DNA oligomers were hydrophobic forces, van der Waals forces and H-bonding. This study provides detailed information about the complexation of Harmine with DNA and also its specificity towards base pairs which provide a deep insight into the mechanism of action of drug.

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