

Interaction of Resveratrol and Genistein with Nucleic Acids

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Resveratrol (RES) and genistein (GEN) are the dietary natural products known to possess chemopreventive property and also the ability to repair DNA damage induced by mutagens/carcinogens. It is believed that the therapeutic activity of these compounds could be primarily due to their interaction with nucleic acids but detailed reports are not available. We here explore the interaction of these drugs with nucleic acids considering DNA and RNA as a potential therapeutic target. The interaction of RES and GEN has been analysed in buffered solution with DNA [saline sodium citrate (SSC)] and RNA [tris ethylene diamine tetra acetic acid (TE)] using UV-absorption and Fourier transform infrared (FTIR) spectroscopy. The UV analysis revealed lesser binding affinity with nucleic acids at lower concentration of RES (P/D = 5.00 and 10.00), while at higher drug concentration (P/D = 0.75, 1.00 and 2.50) hyperchromic effect with shift in the λ_{\max} is noted for DNA and RNA. A major RES-nucleic acids complexes was observed through base pairs and phosphate backbone groups with $K = 35.782 \text{ M}^{-1}$ and $K = 34.25 \text{ M}^{-1}$ for DNA-RES and RNA-RES complexes respectively. At various concentrations of GEN (P/D = 0.25, 0.50, 0.75, 1.00 and 2.50) hyperchromicity with shift in the λ_{\max} from 260 \rightarrow 263 nm and 260 \rightarrow 270 nm is observed for DNA-GEN and RNA-GEN complexes respectively. The binding constant (from UV analysis) for GEN-nucleic acids complexes could not be obtained due to GEN absorbance overlap with that of nucleic acids at 260 nm. Nevertheless a detailed analysis with regard to the interaction of these drugs (RES/GEN) with DNA and RNA could feasibly be understood by FTIR

spectroscopy. The NH band of free DNA and RNA which appeared at $3550\text{-}3100 \text{ cm}^{-1}$ and $3650\text{-}2700 \text{ cm}^{-1}$ shifted to $3450\text{-}2950 \text{ cm}^{-1}$ and $3550\text{-}3000 \text{ cm}^{-1}$ in DNA-RES and RNA-RES complexes respectively. Similarly shifts corresponding to $3650\text{-}3100 \text{ cm}^{-1}$ and $3420\text{-}3000 \text{ cm}^{-1}$ have been observed in DNA-GEN and RNA-GEN complexes respectively. The observed reduction in NH band of free nucleic acids upon complexation of these drugs is an indication of the involvement of the hydroxyl (OH) and imino (NH) group during the interaction of the drugs and nucleic acids (DNA/RNA) through H-bonded formation. The interaction of RES and GEN with bases appears in the order of $G \geq T > C > A$ and $A > C \geq T > G$. Further interaction of these natural compounds with DNA and RNA is also supported by changes in the vibrational frequency (shift/intensity) in symmetrical and asymmetrical stretching of aromatic rings of drugs in the complex spectra. No appreciable shift is observed in the DNA and RNA marker bands, indicating that the B-DNA form and A-family conformation of RNA are not altered during their interaction with RES and GEN.

Keywords: DNA, FTIR, Genistein, Resveratrol, RNA, UV-absorption

Introduction

RES (3,5,4'-trihydroxy stilbene) and GEN (5,7,4'-trihydroxy isoflavone) are synthesized by a wide variety of dietary plant species such as grapes, mulberries and peanuts (Jang *et al.*, 1997) and in dietary isoflavones such as beans and legumes (Fotsis *et al.*, 1993) and it has been of immense interest to study their activity during the last ten years. Numerous studies have focused on the mechanism by which these can inhibit cancerous cell growth (Adlercreutz and Mazur 1997; Jang and Pezzuto 1999). The ever growing realization of the variety of biochemical roles of nucleic acids (Helen *et al.*, 1998) in all biological organisms is leading to an increasing appreciation

Abbreviations: RES, Resveratrol; GEN, Genistein; FTIR, Fourier transform infrared; P/D, phosphate/drug; TE, tris ethylene diamine tetra acetic acid; SSC, saline sodium citrate; NaOH, sodium hydroxide; OH, hydroxyl; NH, imino; H-bond, hydrogen bond; PO_2^- , phosphate anion

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that cellular nucleic acids provide inviting targets for drugs (Johnson *et al.*, 2003) in the treatment of both infectious and chronic diseases. Most of the antitumor/antimicrobial agents exert their effect by inhibiting DNA replication and RNA processing leading to cell death (Natarajan *et al.*, 1999; Chandrasekar *et al.*, 2000). Binding of these agents to DNA can cause any alteration in DNA structure (Natarajan *et al.*, 1998), leading to impaired gene expression and alter the biological functions and may inherit to offspring. Recently, RNA has been recognized as a novel therapeutic target (Tor 1999) and small molecules are found to inhibit transcription, regulation, translation and viral replication and thus modify cellular process.

Currently extended work is in progress in designing novel therapeutic agents at molecular level to combat increase in resistance problem and the specificity for a vast range of target such as nucleic acids other than the cell membrane. Understanding the mechanism of interaction of RES and GEN with nucleic acids and the effects they produce on the molecular geometry and conformation of nucleic acids has been a subject of considerable interest and assumes tremendous significance in developing them as therapeutic agents targeted to DNA and RNA.

RES and GEN gain structural similarities in their phenolic rings, which is indispensable for estrogen receptor binding (Leclercq and Heuson, 1979) and the hydroxyl group in 4th position in them is known for their antioxidant activity. They appear to block the cell cycle at the transition S → G₂/M and G₂/M (Nishino and Aoike, 1993; Delmas *et al.*, 2002) in a dose dependent manner. Reports (Ahmad *et al.*, 2000) have shown that RES can result in DNA strand breaks in the presence of Cu (II), whereas GEN (at similar concentration of RES) can mediate DNA strand breaks by H₂O₂/Cu (II) thus exerting their influence on oxidative DNA damage (Win *et al.*, 2002). RES is found to mediate the relaxation of pBR322 (Fukuhara and Miyata, 1998), inhibit DNA polymerase (Sun *et al.*, 1998) and ribonucleotide reductase (Fontecave *et al.*, 1998) etc. However detailed information on the mode of their interaction and the type of adducts formed with nucleic acids has not been well understood and hence the present analysis.

The absorption spectrum hints at the interaction of these drugs to DNA and RNA bases. While the FTIR analysis is indicative of the preferential binding of drugs and nucleic acids through H-bonding (OH of drugs with NH of the bases). This study possibly explains the interaction of RES/GEN in identifying the common binding motif of nucleic acids, which might pave the way for the development of novel therapeutics with alternative targets in cancer and infectious diseases.

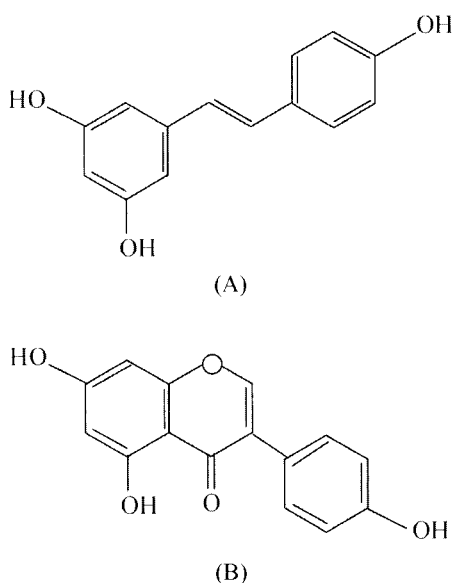


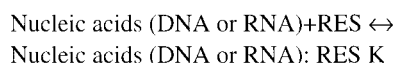
Fig. 1. Structure of (a) Resveratrol (3,5,4'-trihydroxy stilbene) and (b) Genistein (5,7,4'-trihydroxy isoflavone).

sodium citrate, pH 7.0) and 1X TE (10 mM Tris and 0.1 mM EDTA, pH 7.4) buffers respectively and they were used for UV-absorption study. For FTIR analysis, Herring's sperm DNA (not highly polymerized) (HiMedia, Mumbai, India) and Torula yeast RNA were used.

RES and GEN RES and GEN (Fig. 1) were purchased from ICN Biomedicals USA. RES and GEN were dissolved in cold 3 mM NaOH to make the stock of 1 mM. These were added to the reaction mixtures in buffered solutions at specific concentrations, where the volume of the stock solutions added to the mixture did not alter the pH of the solution (Ahmad *et al.*, 2000).

UV-absorption RES was added to DNA (20 µg) and RNA (16 µg) discretely at different P/D ratios such as 0.75, 1.00, 2.50, 5.00 and 10.00 and GEN was added to DNA and RNA at P/D ratios 0.25, 0.50, 0.75, 1.00 and 2.50. The final volume was made up to 1 ml using 1X SSC and 1X TE buffer respectively. The samples were further incubated overnight at 37°C. After incubation the samples were then scanned between 220-370 nm for RES and 210-300 nm for GEN using UV-Visible spectrophotometer (Shimadzu UV-300, Japan). The absorbance changes in complexes were recorded and compared with that of free drugs, free DNA and RNA respectively.

The binding constant for RES-nucleic acids (DNA or RNA) complexes was carried out (Stephanos 1996; Djoman *et al.*, 1998) in assumption with only one type of interaction (H-bonding) between nucleic acids (DNA or RNA) and RES, the following equation was thus established.



$$K = \frac{[\text{Nucleic acids (DNA or RNA): RES}]}{[\text{Nucleic acids (DNA or RNA)}][\text{RES}]}$$

Materials and Methods

DNA and RNA Calf thymus DNA (Sigma, St. Louis, USA) and Torula yeast RNA (SRL, India) were prepared as a stock concentration of 0.01% in 1X SSC (0.15 M NaCl and 0.015 M

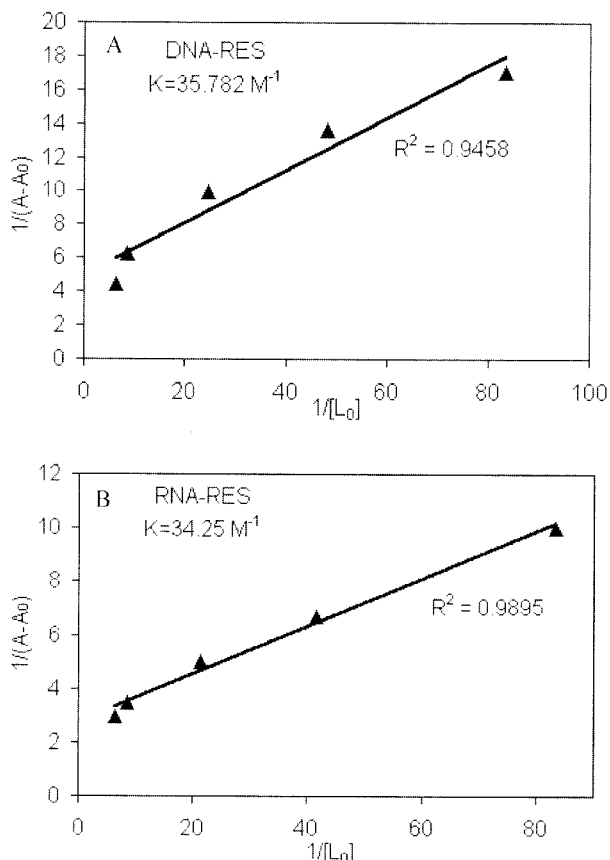


Fig. 2. The double reciprocal plot of $1/(A-A_0)$ vs $1/[L_0]$, for (A) DNA-resveratrol and (B) RNA-resveratrol complexes, where A_0 = absorbance (260 nm) of free nucleic acids (DNA or RNA), $[L_0]$ = different drug concentration of RES.

The double reciprocal plot was derived for DNA-RES and RNA-RES complexes according to the formula $1/(A-A_0)$ vs $1/[L_0]$, where A = absorbance of the nucleic acids at different drug concentration $[L_0]$, A_0 = absorbance (260 nm) of free nucleic acids (DNA or RNA), $[L_0]$ = different drug concentration of RES. Also the percentage of binding activity was calculated (with an error of $\pm 2\%$) for DNA-RES, RNA-RES complexes by computing the formula $100(A_{260} - A_{260}^0/A_{260}^0)$; where A_{260} , absorbance (at 260 nm) value obtained for drug complexation with nucleic acids at particular concentration; A_{260}^0 , absorbance (at 260 nm) of free nucleic acids (Johnson *et al.*, 2003).

FTIR spectra RES and GEN were complexed with DNA and RNA separately in 1 : 2 ratio. FTIR analysis (Burker IFS 66V, Germany) was performed for free drugs, free nucleic acids and complexes with respect to our published protocol (Johnson and Malathi, 2004).

Results and Discussion

UV-absorption The UV-spectra of DNA and RNA in the presence of RES and GEN at varying concentrations are

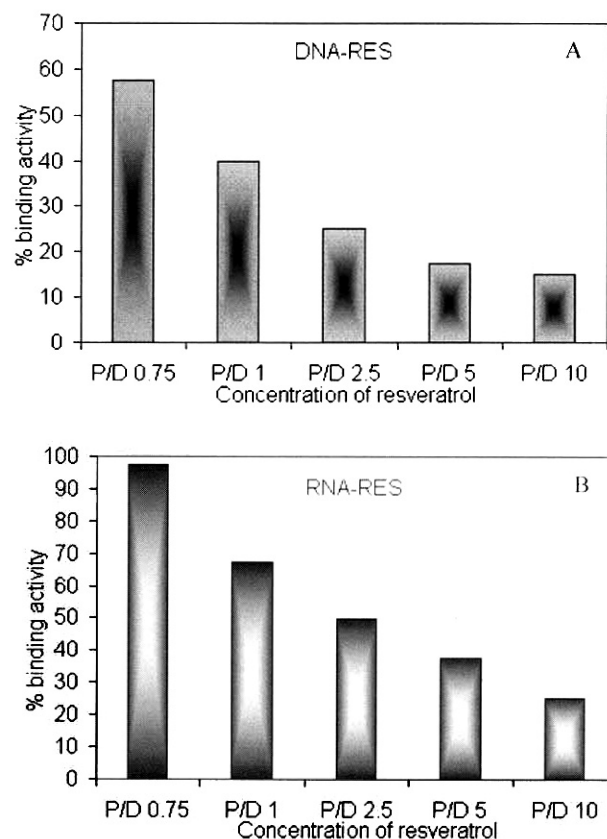


Fig. 3. Percentage of binding activity of resveratrol with (A) DNA and (B) RNA. The binding affinity increases with regard to increasing concentration of the drug and correlates with that of the binding constant that has been derived for DNA-RES and RNA-RES complexes as indicated in Fig. 2 (A) and (B).

recorded. The binding constant and the percentage of binding activity/affinity for nucleic acids-RES complexes are represented in Figs. 2 and 3. The calculation of either binding constant or binding activity has been hindered for nucleic acids-GEN complexes due to absorbance overlap of the drug molecule with that of the nucleic acids. However the changes observed in the characteristic, absorption maximum (λ_{max}) for nucleic acids (DNA/RNA) (260 nm) with GEN (265 nm) at various drug concentrations (P/D = 0.25, 0.50, 0.75, 1.00 and 2.50) and hyperchromicity are observed in the DNA peak (Table 1), suggesting the probable interaction of GEN with nucleic acids.

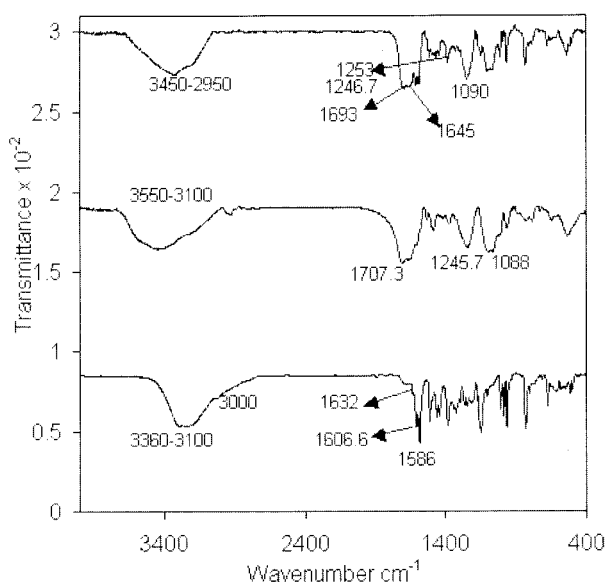
There was a lesser binding affinity observed for RES at its lower concentration (P/D = 5.00 and 10.00) with DNA and RNA during complexation. This could be understood from the overall binding constant derived for DNA-RES and RNA-RES complexes, indicating a weak complexation in aqueous solution. This is mainly because of the negative charge of both nucleic acids (PO_2^-) and $\cdot OH$ group of RES, causing destabilization of the complexes. The double reciprocal plot obtained for nucleic acids-RES complexes is linear and correlating with that of the calculated binding activity (Fig.

Table 1. UV absorption spectra of DNA and RNA in the presence of genistein

	Spectral changes observed in complexes									
	DNA-Genistein					RNA-Genistein				
P/D ratios	0.25	0.50	0.75	1.00	2.50	0.25	0.50	0.75	1.00	2.50
Shift in absorbance (from 0.4 O.D) at 260 nm	1.63	1.53	1.42	1.25	1.03	1.85	1.77	1.65	0.99	0.76
Shift in λ_{\max} (indicated with absorbance) from 260 nm	1.70/263	1.57/263	1.46/263	1.28/263	1.13/263	1.98/270	1.88/270	1.81/270	1.65/270	1.21/270

Free DNA/RNA = 0.4 O.D/260 nm

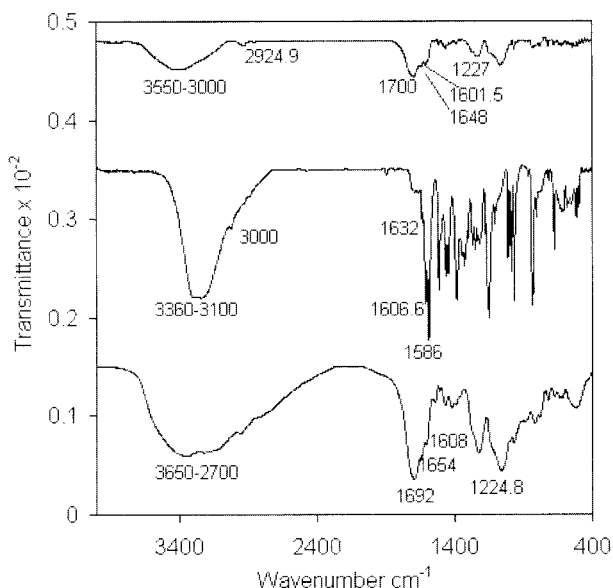
Free Genistein = 0.86 O.D/265 nm

**Fig. 4.** FTIR spectra of free resveratrol, free DNA and DNA-resveratrol complex are depicted at the bottom, middle and top of the figure respectively.

3.). Furthermore at higher drug concentrations (P/D = 0.75, 1.00 and 2.50), hyperchromicity (0.4 \rightarrow 0.63/0.79 and 0.56/0.67) with shift in the λ_{\max} (260 \rightarrow 315/300 nm) was observed in DNA-RES and RNA-RES complexes respectively, suggesting a plausible binding of RES with the nucleic acids.

Thus it could be understood from the above that the destabilization of nucleic acids structure brought about by binding of RES and GEN is either by forming adducts with the bases or by partial stacking between the bases. The UV-absorption study also indicates the possibility of more than one type of binding and hence the binding constant in the case of nucleic acids-GEN complexes could not be derived in addition to the absorbance overlap. However the detailed interaction of these drugs with DNA and RNA has been enlightened by FTIR analysis.

FTIR analysis FTIR spectroscopic method is employed to study the interaction of DNA/RNA-RES and GEN complexes wherein the changes observed in vibrational frequencies of

**Fig. 5.** FTIR spectra of free RNA, free resveratrol and RNA-resveratrol complex are depicted at the bottom, middle and top of the figure respectively.

their functional groups are explored in detail (Figs. 4-6 and Table 2).

DNA-RES and GEN interaction The infrared spectrum of DNA-RES complex (Fig. 4.) has offered an evidence for the direct binding of RES to DNA. The vibrational frequencies of the NH and OH group of free DNA appeared around 3550-3100 cm^{-1} , has shifted into 3450-2950 cm^{-1} in the complex. The broadening in free DNA band is mainly attributed to the intramolecular H-bonding and the observed reduction in NH band on drug interaction is indicative of the interaction of RES with DNA bases. The interaction of RES could also be envisaged from the observed change mainly in the vibrational frequency of OH (ν_{OH}) of RES appeared around 3360-3100 cm^{-1} has changed into 3550-3000 cm^{-1} in DNA-RES complex. Thus the vibrational frequencies of NH band in DNA and OH in drug has changed in complex, obviously representing the effective interaction of drug OH with NH of DNA bases which could be possible through H-bond interaction.

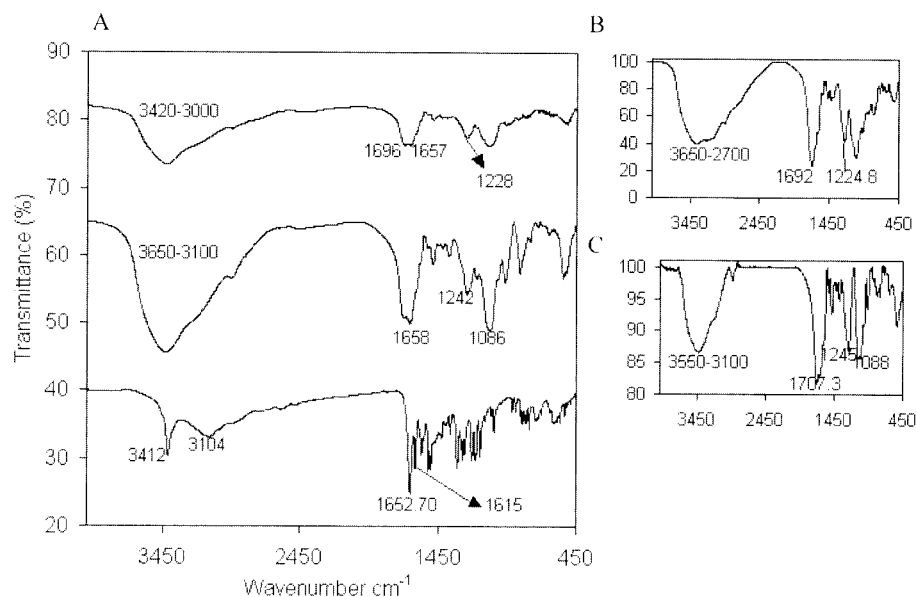


Fig. 6. FTIR spectra of (a) free genistein, DNA-genistein and RNA-genistein complexes are depicted at the bottom, middle and top of the figure respectively (b) free RNA (c) free DNA.

Table 2. The vibrational frequencies of major functional groups in free drugs, DNA, RNA, DNA-drugs and RNA-drugs complexes

Free drugs, free DNA, free RNA and complexes	Observed changes in the vibrational frequency of the functional groups and bases (cm^{-1})								
	OH	C=O	NH	PO_2^-	A	G	C	T	U
Free Resveratrol	3360-3100	---	---	---	---	---	---	---	---
Free Genistein	3412-3104	1652.7	---	---	---	---	---	---	---
Free DNA	3550-3100	1707.3	3550-3100	1245.7, 1088	1605.5	1707.3	1486	1656	---
Free RNA	3650-2700	1692	3650-2700	1224.8	1608	1692	1488	---	1654
DNA-Resveratrol	3450-2950	1693	3450-2950	1246.7, 1253	1603	1693	1490	1645	---
DNA-Genistein	3650-3100	1658	3650-3100	1086, 1242	1594	1707.3	1490	1658	---
RNA-Resveratrol	3550-3000	1700	3550-3000	1227	1601.5	1700	1483	---	1648
RNA-Genistein	3420-3000	1696	3420-3000	1228	1620	1696	1487	---	1657

The vibrational frequency of C = O of free DNA at 1707.3 cm^{-1} has shifted to 1693 cm^{-1} in the complex. Further indication for H-bonding interaction of RES with DNA bases such as G-C and A-T comes from major spectral changes of DNA in-plane vibrations in the region of $1707\text{-}1400 \text{ cm}^{-1}$ (Alex and Dupuis 1989). The band at 1707.3 cm^{-1} (G, T) related to mainly guanine and 1656 cm^{-1} (T, G, C) mainly for thymine (Ahmad *et al.*, 2003) shifted towards lower frequency with the change in intensity (not measured) in complex at 1693 and 1645 cm^{-1} respectively. The cytosine band at 1486 cm^{-1} (C, G) in free DNA shifted to 1490 cm^{-1} in complex. In contrast, only minor change is observed in the band for adenine from 1605.5 cm^{-1} (Ahmad *et al.*, 2003) to 1603 cm^{-1} upon drug complexation but a profound intensity variation is observed (not calculated) for adenine band in complex when compared to the free form. Thus the drug shows a preferential binding affinity for DNA bases and the order could be visualized as $G \geq T > C > A$.

Additional evidence for DNA-RES interaction is obtained

from the spectral shifting of RES vibrational frequencies upon DNA binding. The frequency of =C-H stretching appeared for free drug at 3000 cm^{-1} (Soni and Chawla 1994), has merged in the region between $3350\text{-}3000 \text{ cm}^{-1}$ in the complex is suggestive of interaction of RES with DNA. The band at 1632 cm^{-1} of free drug related to the unsymmetrical C = C stretching has changed into 1693 cm^{-1} in the complex. The out of plane C-H bending at 805 cm^{-1} (Silverstein and Webster, 1997) in free drug has shifted to 800 cm^{-1} in the complex, with a profound change in the intensity. Some minor changes (shifting/mainly intensity variation) are also observed in the free drug at 1606.6 cm^{-1} (symmetrical C = C stretching), 1586 and 1462 cm^{-1} (C-C ring stretching), 1350 cm^{-1} (in plane OH bend), 1217 cm^{-1} (C-O stretching) and 675 cm^{-1} (out of plane ring C-C bend) (Silverstein and Webster, 1997).

The phosphate anion (PO_2^-) stretching band of free DNA at 1245.7 cm^{-1} and 1088 cm^{-1} (Ahmed Ouameur 2003) exhibited splitting into 1246.7 and 1253 cm^{-1} in asymmetric and 1090 cm^{-1} in symmetric vibration with minor intensity changes in

the complex. Also the IR marker bands at 883.6 cm^{-1} (sugar-phosphate stretch) and 823.5 cm^{-1} (phosphodiester mode) present in free DNA (Ahmad *et al.*, 2003) have not appreciably shifted (only intensity variation is observed). The minor perturbations in the PO_2^- backbone geometry indicate that the DNA remains in the B-family conformation. The shift of the guanine band at 1707.3 to 1693 cm^{-1} in complex could be mainly due to the H-bonding interaction involving the drug OH and NH of bases.

The infrared spectrum of DNA-GEN complex (Fig. 6.) implies that the vibrational frequency of NH/OH of free DNA at $3550\text{--}3100\text{ cm}^{-1}$ and the vibrational frequency of OH of GEN at $3412\text{--}3104\text{ cm}^{-1}$ has shifted towards $3650\text{--}3100\text{ cm}^{-1}$ in the complex. This suggests that the NH of free DNA bases and OH of GEN are involved in mutual H-bonding interaction. This is further supported by the interesting observation that the frequency vibration of DNA bases, free adenine at 1605.5 cm^{-1} has shifted to 1594 cm^{-1} in DNA-GEN complex, hints at the greater affinity of adenine to bind with GEN. The cytosine and thymine of free DNA at 1486 and 1656 cm^{-1} has shifted towards 1490 and 1658 cm^{-1} respectively and guanine peak exhibited no such variation in the complex. The affinity of GEN to DNA bases is noticed in the order of $A > C \geq T > G$ and provides a profound binding of GEN with DNA bases.

Besides the functional OH group of drug, the symmetrical and asymmetrical stretching is also essential in the binding of aromatic ring with DNA. The vibrational frequency of $\text{C}=\text{O}$ of DNA and drug appears at 1707.3 and 1652.7 cm^{-1} has shifted slightly to 1658 cm^{-1} in the complex. The frequency vibration of $\text{C}=\text{C}$ stretching of free drug at 1615 cm^{-1} has disappeared and $\text{C}-\text{O}-\text{C}$ stretching vibration at $1307\text{--}1170\text{ cm}^{-1}$ (Soni and Chawla, 1994) exhibited the shift towards $1320\text{--}1171\text{ cm}^{-1}$ upon complexation. The $\text{C}-\text{O}$ stretching vibrational frequency of free drug at $1260\text{--}1000\text{ cm}^{-1}$ (Silverstein and Webster, 1997) also shifted to $1242\text{--}964\text{ cm}^{-1}$ and gained intensity variation (not measured). The relative out of plane C-H bend at 790 cm^{-1} (Silverstein and Webster, 1997) has reduced to 810 cm^{-1} in the complex. These changes in stretching and bending vibrational frequency of GEN additionally support the H-bonding interaction of drug with DNA. Minor variation in PO_2^- symmetric and asymmetric vibration at 1088 to 1086 cm^{-1} and 1245.7 to 1242 cm^{-1} , IR marker band (sugar phosphate stretch) 883 to 885 cm^{-1} in DNA-drug complex are observed. This indicates that the B-DNA family conformation is maintained in DNA-GEN complex.

RNA-RES and GEN interaction The FTIR spectrum of RNA-RES complex (Fig. 5.) reveals that the NH and OH group vibrational frequencies of free RNA which appeared in the region $3650\text{--}2700\text{ cm}^{-1}$ has been altered upon drug interaction with RNA. It was observed that the broad band of NH of the free RNA was drastically reduced in the complex and appeared around $3550\text{--}3000\text{ cm}^{-1}$ with a corresponding

change in the OH vibrational frequency (ν_{OH}) of free drug, which appeared in the region $3360\text{--}3100\text{ cm}^{-1}$. This is indicative of the loss of intramolecular H-bonding of RNA bases due to the interaction of drug OH with NH of RNA through H-bond formation.

Interestingly the changes in the RNA-in-plane vibrations of bases (G, C, A, U) and the PO_2^- backbone geometry could be observed from the shift in the bands at 1692 (G, U or mainly G) to 1700 cm^{-1} , 1654 (U, G, A, C or mainly U) (Djoman *et al.* 1998) to 1648 cm^{-1} , 1608 (A) to 1601.5 cm^{-1} , 1488 (C,G or mainly C) to 1483 cm^{-1} and 1224.8 (PO_2^- stretch) to 1227 cm^{-1} . This supports the interaction of drug with RNA bases, exhibiting a minor alteration in the sugar-phosphate geometry. We also observed minor intensity variations in the RNA marker bands at 860 , 808 and 800 cm^{-1} , due to ribose-phosphate stretching modes (Brahms *et al.*, 1974) in the complex. It is also observed that the ribose ring vibrations at 1105 cm^{-1} (C-O stretch), 1063 cm^{-1} (C-O and C-C stretching), 996 and 976 cm^{-1} (C-C stretching) in the free RNA (Djoman 1998) showed some minor perturbations upon RES interaction.

Additional evidence for RNA-RES interaction is offered from the spectral shifting of the RES vibrational frequencies. The frequency of $=\text{C}-\text{H}$ of drug at 3000 cm^{-1} has shifted to 2924.9 cm^{-1} , suggesting that $=\text{C}-\text{H}$ of drug is also involved in interaction with RNA, where a corresponding change in the $\text{C}=\text{O}$ vibrational stretching frequency of free RNA at 1692 cm^{-1} has shifted to 1700 cm^{-1} in complex. The band at 1632 cm^{-1} of free drug related to the unsymmetrical $\text{C}=\text{C}$ stretching has shifted towards 1694 cm^{-1} in the complex. The out of plane C-H bending at 805 cm^{-1} in free drug has shifted into 800 cm^{-1} and changes (shifting/mainly intensity variation) were also observed in the free drug at 1606.6 (symmetrical $\text{C}=\text{C}$ stretching) to 1601 cm^{-1} , 1586 and 1462 cm^{-1} (C-C ring stretching), 1350 cm^{-1} (in plane OH bend), 1217 cm^{-1} (C-O stretching) and 675 cm^{-1} (out of plane ring C-C bend) also showed minor variation in the complex.

Though we observed a strong interaction of RES with RNA, the structure has not been changed and RNA remains in A-conformation, as no changes are observed in the IR marker bands of RNA such as 1227 cm^{-1} (PO_2^- stretch), 1700 cm^{-1} (G, U) (Brahms *et al.*, 1974) and 860 , 808 and 800 cm^{-1} (sugar-phosphate vibration) and hence the RNA remains in A-family structure in RNA-RES complexes.

The IR spectral feature of RNA-GEN binding is shown in Fig. 6. Evidence for RNA-drug interaction comes from NH/OH group vibrational frequency of free RNA at $3650\text{--}2700\text{ cm}^{-1}$ and OH of free GEN at $3412\text{--}3104\text{ cm}^{-1}$ showed shift towards $3420\text{--}3000\text{ cm}^{-1}$ in the complex. Similar result was observed as in RES complex where there is a decrease in vibrational frequency of NH and OH functional groups. This is obviously due to reduction in the intramolecular H-bonding of RNA bases and this indicates that the functional OH group of GEN interacts with free RNA by H-bond formation between GEN and RNA.

Evidence for direct drug binding to bases comes from the

major spectral changes of A (1608 cm^{-1}) to 1620 cm^{-1} , G (1692 cm^{-1}) to 1696 cm^{-1} , mainly C or C, G (1488 cm^{-1}) to 1487 cm^{-1} and U (1654 cm^{-1}) to 1657 cm^{-1} is observed. The PO_2^- stretching band at 1224.8 cm^{-1} has shown reduction in intensity (not measured) and shifted towards 1228 cm^{-1} in the complex. The relative minor intensity variation of RNA marker bands, ribose ring vibration at C-O stretch and C-C stretch of complex whereas the vibrational frequencies remains unaltered as in case of RNA-RES complex.

The carbonyl (C=O) vibrational frequency ($\nu_{\text{C=O}}$) of RNA and GEN from 1692 cm^{-1} and 1652.7 cm^{-1} has shifted to 1696 cm^{-1} in the complex respectively. It has been suggested that the RNA-GEN interaction was also observed from the changes in stretching vibrational frequencies of GEN. The C=C stretching (1615 cm^{-1}), C-O stretch (1260-1000 cm^{-1}), out of plane C-H bend (808 cm^{-1}) of a free drug have shown minor intensity variation and there is no appreciable change in vibrational frequency upon complexation. Obviously major spectral shifting of RNA-GEN complex is due to H-bond formation between OH of drug with NH of RNA bases as in case of RNA-RES complex. However there is minor intensity variation in IR marker band and no alteration in ribose-phosphate vibration at 860, 808 and 800 cm^{-1} in RNA-GEN complex. It is worth mentioning to note that the functional OH group of GEN is involved in H-bond formation with bases especially guanine of RNA. A minor variation noted in the RNA marker band suggests that RNA remains in A-family conformation.

Taken together the present study using UV- absorption spectral analysis hints the binding of RES/GEN with DNA and RNA is exhibited by hyperchromicity with slight shift in wavelength thereby destabilizing the DNA stacking and adduct formation with RNA bases. This is further evidenced by FTIR analysis. The OH group of RES and GEN is expected to be favored and primary determinant for its interaction with nucleic acids. The complexation of RES and GEN occurred with G-C, A-T and G-C, A-U of DNA and RNA bases respectively where the NH and OH groups of bases in nucleic acids (DNA/RNA) and drugs are mutually involved in H-bonding. This was observed from the reduction in the broadening of NH band of DNA and RNA upon drugs complexation. This interaction could also be presumed from RES = C-H and GEN C=O functional groups. However no alteration was observed in the ribose-phosphate backbone geometry and in the marker bands. Hence there is no alteration in DNA (B-conformation) and RNA (A-conformation). Generally IR spectra for DNA and RNA are taken in solution but in our case IR spectra for DNA-drug and RNA-drug complexes were taken in solid state using KBr pellet (Johnson and Malathi, 2004). Hence there is minor variation in the IR transmittance for PO_2^- stretch and other marker bands by 10-20 cm^{-1} . The result from our report denotes that RES and GEN binds to nucleic acids through H-bond formation and this has triggered a renewed interest in studying their delivery to the targets, especially the spotlight

could be focused to study the structure and functional relationship of RES/GEN with nucleic acids which might be helpful for targeted drug delivery or improved therapeutic interventions.

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