phosphate buffer pH 7.5 in a final reaction volume of 1ml at room temperature .Drug solutions were prepared in the same buffer. Fluorescence measurements were carried out on a Shimadzu Spectrofluorimeter model RF-5301PC (Shimadzu, Japan) equipped with a 150 W Xenon lamp and a slit width of 10 nm at 298K. The fluorescence was recorded in wavelength region 300-400 nm after exciting the protein at 280 nm. The slits were set at 10 nm for excitation and emission. The path length of the sample was 1 cm. The data was analyzed by stern-Volmer equation

Stern- Volmer Constant

The fluorescence quenching was analyzed by the Stern-Volmer equation

$$\frac{F_o}{F} = 1 + Ksv\,[Q]$$

Where F_0 and F are the fluorescence intensities in the absence and presence of quencher respectively, Ksv the stern-volmer quenching constant and [Q] is the concentration of the quencher.

Determination of binding constant [K] and number of binding sites (n)

When small molecules binds independtly to set of equivalent sites on a macromolecules, the equilibrium between free and bound molecules is given by the following equation [20,13]

$$Log\frac{(F_0 - F)}{F} = LogK + n\,Log[Q]$$

Where K and n are the binding constant and number of binding sites respectively thus a plot of Log [(Fo-F)/F versus [Q] can be used to determine K as well as n.

UV spectra of cystatin in the presence of antidepressant

The UV measurement of brain cystatin in the presence and absence of haloperidol were made in the range of 200-300 nm and the inhibitor (Cystatin) concentration was fixed at 1µM while the drug concentration was varied for different drugs to different extent. Absorption spectra were recorded on a double beam Shimadzu UV-vis spectrophotometer UV-1700 using a cuvette of 1 cm path length.

Activity measurement of brain cystatin in the presence of drug haloperidol

The inhibitory activity of the purified inhibitor (BC) under native conditions was assessed by its ability to inhibit caseinolytic activity of papain by the method of *Kunitz [Kunitz (1947)]*. The inhibitor (1 µM) was incubated with increasing concentrations of drugs at 25^0C for 30 min before the activity was measured. Activity of untreated BC was taken as 100%.

3. Result

Fluorescence spectra of Haloperidol with brain cystatin 1µM of BC treated with increasing concentration of Haloperidol (0.02-0.1µM) and was subjected to fluorescence spectroscopy to assess the effect on conformation. The protein samples were excited at 280nm and emission range was 300-400nm. Haloperidol at 0.02µM did not induce any change in emission λ_{max} however 68% decline in fluorescence intensity was observed. Beyond this concentration (0.08µM) profound decline in fluorescence intensity (75%) was observed. Significant quenching in parent protein was observed (95%) when cystatin was incubated with 0.1µM of Haloperidol. [Fig-3].

Percent decrease in tryptophan fluorescence in the presence of Haloperidol with cystatin

BC alone fluorescence	Drug alone	0.02 µM	0.04 µM	0.06 µM	0.08 µM	0.1 µM
100	98	68	77	69	75	95

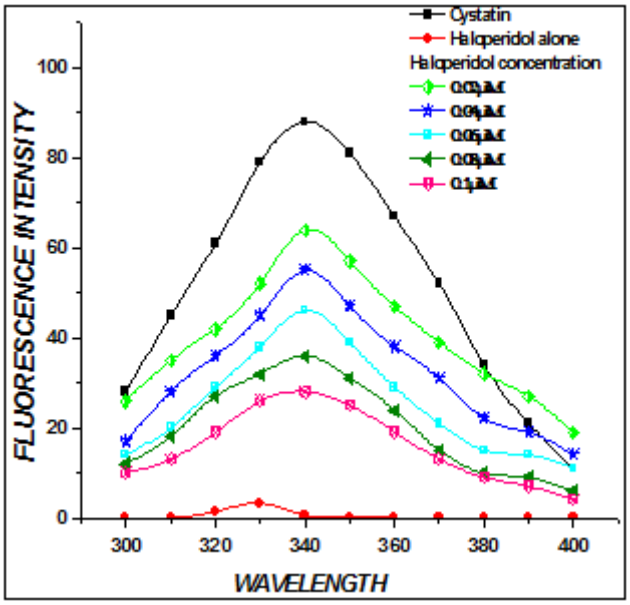

Figure 3: Fluorescence spectra of Cystatin in the presence and absence of Haloperidol

Cystatin (1µM) was incubated with various concentration of Haloperidol varying from 0.02µM to 0.1µM for 30min. The fluorescence was recorded in wavelength region 300-400 nm after exciting the protein solution at 280 nm

Stern–Volmer constant

For fluorescence quenching, the decrease in intensity is usually described by the well-known Stern–Volmer equation The value of Ksv was found to be 15 x10 ^{6} mol $^{-1}$ for Haloperidol *[Table-1]*.

Determination of binding constant (K) and number of binding sites (n)

The value was determined as described earlier in amytriptyline section. The binding constant and the number of binding sites was found to be 7.988 x10^{6} mol $^{-1}$ and 1.1 respectively *[Table-1]*

ΔG^0 of interaction between Haloperidol with Cystatin

The forces of interaction between drugs and biomolecules include hydrophobic force, electrostatic interactions, vander Waals interactions and hydrogen bonds. In order to identify the interacting forces between amytriptyline with cystatin, the thermodynamic parameters i.e. free change (ΔG^0) of the

interactions were calculated from the following equations: *[Table-1]*

$$\Delta G^{\circ} = -RT\,Ink\,(KJ/mol)$$

Free energy change (ΔG^0), of the interactions was calculated from the equations was -39.37 KJ/ mol . *[Table-1]* indicating the reaction to be spontaneous

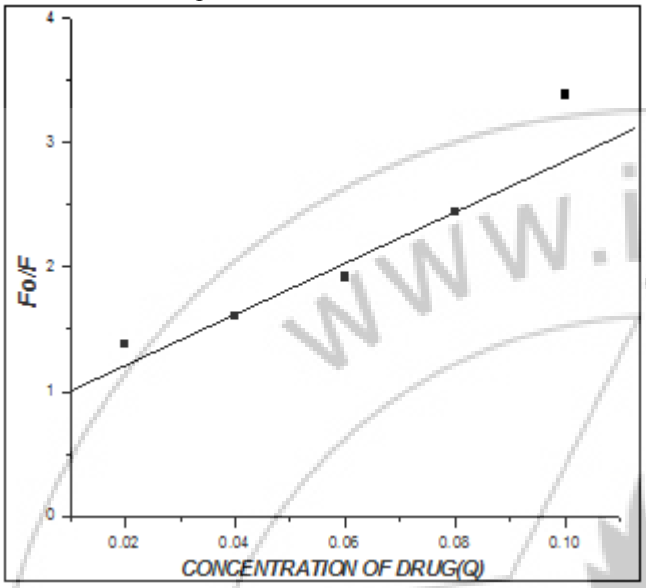

Figure 4: Stern-Volmer plots for the binding of BC to haloperidol at different concentrations at 298 K

Table 2: Different Parameter of the Drugs Obtained By Stern Volmer Equation for Interaction with Cystatin

Drug Parameter	K_{SV} (Stern-volmer Constant) Mol^{-1}	K (Binding constant) Mol^{-1}	N (number of binding sites)	ΔG^0 (Free energy change) KJ/mol
HALOPERIDOL	15 x 10^6	7.988 x 10^6	1.1	-39.377

Absorption spectra of cystatin in the presence and absence of Haloperidol

The interaction between Haloperidol with cystatin was studied from UV-VIS absorption spectral data cystatin concentrations was fixed at 1µM while the haloperidol concentration was varied from 0.02µM-0.1µM. Absorption spectra of native cystatin and in presence of Haloperidol were recorded in the range of 200-300 nm. The UV absorption intensity of cystatin increased with the variation of Haloperidol concentration. UV absorbance spectra of cystatin, Haloperidol and their complexes are shown in *[Fig-5]* BC showed peak in the region 200-210 nm while on complexation with Haloperidol profound changes were introduced and there was peak shift of 30nm (red shift) in the range of 200-300 nm, with deep enhancement in absorbance. Shows that some complex formation might be taking place between haloperidol and cystatin[14,15]

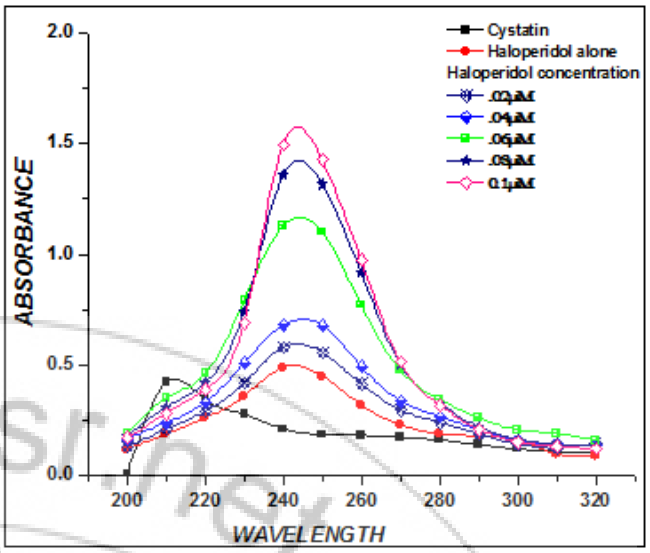

Figure 5: UV-vis spectra of Cystatin in the presence and absence of Haloperidol

Cystatin concentrations was fixed at 1µM while the Haloperidol concentration was varied from 0.02µM-0.1µM. Absorption spectra of native BC in the presence and absence of Haloperidol were recorded in the range of 200-300 nm.

Inhibitory activity of Cystatin in the presence of Haloperidol

The obtained data indicates that the inactivation of Brain cystatin by Haloperidol is concentration dependent. 1µM cystatin was incubated with increasing concentrations of the drug (0.02-0.1 µM) in 50 mM sodium phosphate buffer pH 7.5 at room temperature for 30 min and its inhibitory activity was determined by caseinolytic assay of papain [26]. The activity of native cystatin was taken as 100%. The exposure of 1µM of cystatin to varying concentrations of Haloperidol resulted in remarkable loss in its antiproteolytic activity. At concentration as low as 0.06µM haloperidol showed 42% loss in activity of cystatin .On interaction with 0.02 µM haloperidol ,29% loss of BC activity was noticed *[Table-2]*. At 0.1µM drug concentration the inhibitor retained only 36% of its original papain inhibition potential. For total protein fluorescence. The slits were set at 10 nm for excitation and emission. The path length of the sample was 1 cm.

Table 2: Inhibitory Activity of Cystatin In the Presence of Haloperidol

S.No	*Haloperidol concentrations with cystatin*	*% Remaining Inhibitory Activity*
1	Cystatin alone	100
2	Cystatin + 0.02 µM	71 ± 0.938
3	Cystatin +0.04 µM Haloperidol	65 ± 0.915
4	Cystatin +0.06 µM Haloperidol	58 ± 0.920
5	Cystatin +0.08 µM Haloperidol	43 ± 0.770
6	Cystatin +0.1 µM Haloperidol	36 ± 0.911

Changes in the inhibitory activity of cystatin after incubation for 30 min with increasing concentration of Haloperidol. Cystatin (1µM) was treated with varying concentration of Haloperidol (0.02 µM-0.1µM) for 30min in the final reaction volume of 1 ml in 0.05M sodium phosphate buffer pH 7.5.

All data are expressed as mean ± S.E for four different sets of experiments statical significance was conducted employing ONE WAY ANOVA .a probability level of 0.05 was selected showing results are significant.

4. Discussion

Haloperidol It is classified as a highly potent neuroleptic compound possessing a strong activity against delusions and hallucinations, most likely due to an effective dopaminergic receptor blockage in the mesocortex and the limbic system of the brain. The drug is rapidly absorbed. Plasma-levels reach their maximum within 20 minutes after injection. It is known that the distribution, free concentration and the metabolism of various drugs are strongly affected by drug–protein interactions in the blood stream [11,9,16]. This type of interaction can also influence the drug stability and toxicity during the chemotherapeutic process [11]

The Haloperidol - cystatin interaction was chosen as an example of analytical study because of its importance as a common antidepressant. *Sulkowski et al.*[6] investigated the binding interaction of methotexate with BSA by monitoring the changes in the fluorescence emission spectra of the protein in the presence of Methotexate .the results are very similar to our results in which binding of haloperidol with Cystatin has been studied . The binding constant (K) between haloperidol and Cystatin was found to be 7.988 x 10^6 showing strong binding affinity .Haloperidol- cystatin interaction gave one binding site with negative value of ΔG showing the reaction to be spontaneous *[Table-1]*. The interactions between gemcitabine hydrochloride (GEM) and BSA also showed similar results [7].The values of n for GEM- BSA interaction varied from 1.16 to 1.09.

The purified inhibitor was incubated with increasing concentrations of Haloperidol and its antiproteolytic potential was determined. Haloperidol diminished the activity of cystatin with maximum inactivation of the inhibitor at higher concentration within a short span of time *[Table-2]*. At lower concentration of inhibitor inactivation was less significant. Considerable activity (~58%) was retained by cystatin at 0.06 µM of drug concentration. at maximum concentration of the drug (0.1 µM) brain cystatin retained 36% activity. Drug induced inactivation of proteins has been reported earlier for other proteins also like horse liver alcohol dehydrogenase activity which was affected differentially by various drugs. Few like barbital, caffeine and diazepam exerted no effect however chlorpromazine, sulpiride, morphine reduced the activity and phenytoin enhanced ADH activity [24] Absence of any drug induced conformational change in cystatin suggest that inactivation of the inhibitor may be related to subtle changes in the conformation of the protein induced by the drugs. Similar ligand binding functional changes have been shown for isocitrate dehydrogenase [25]

In conclusion from this study binding of haloperidol with cystatin has been established which is expected to open new doors and new avenues in the screening and design of antidepressant drugs that may be of importance in modern medical research. The work shows the side effect of haloperidol which may be further explored for the accurate dose of effective drug. Since, the pharmaceutical firms need standardized screens for protein binding in the first step of new drug design, this kind of study of interaction between brain cystatin with haloperidol would be useful as a model system in pharmaceutical industry, life sciences and clinical medicine.

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