

# HOST-GUEST COMPLEX OF CUCURBITURIL WITH 5-FLUOROURACIL: STRUCTURAL STUDY, EFFECT ON CYTOTOXICITY, AND INTRACELLULAR ROS GENERATION

T. Jaba Priya,<sup>1,\*</sup> R. Wilfred Sugumar,<sup>1</sup>  
M. Harini,<sup>1</sup> and N. Rajendra Prasad<sup>2</sup>

*Original article submitted April 6, 2022*

Cucurbiturils are magnificent macrocycles that are used as precise molecules for the fabrication of supramolecular chemotherapeutic agents through host-guest molecular recognition. In the present work, the host-guest complex of cucurbit[6]uril (CB[6]) with 5-fluorouracil (Flu, an anticancer drug) is synthesized and its structural study was carried out using UV, XRD, and TG/DTA techniques. The UV-Vis spectrum of the inclusion complex exhibited maximum absorbance with a blue shift at 302 nm whereas the maximum absorbance for free CB[6] was observed at 318 nm. This notable shift in wavelength proves the formation of the inclusion complex of CB[6] with fluorouracil. The host-guest complex of fluorouracil with CB[6] shows sharp endothermic peaks at 403.30C with a heat flow of -0.5 mW/mg the peak covers partially an area of 528.2 J/g, The large variations observed in the DSC curves of the complex compared with free host CB[6] confirm the formation of a stable host-guest complex. The XRD pattern of the host-guest complex is different from the parent compound and this reaffirms the formation of host-guest complex. The cytotoxic effect of 5-fluorouracil and the host-guest complex of CB[6] with fluorouracil on KB cells was determined by MTT assay and the cytotoxicity of anticancer drug Flu towards cancer cells significantly increases by encapsulating 5-fluorouracil into the hydrophobic cavity of CB[6], The ROS generation study was carried out by measurement of intracellular ROS using fluorescent and chemiluminescent probe. The ROS generation of anticancer drugs against cancer cells is significantly increased by encapsulating 5-fluorouracil into the hydrophobic cavity of CB[6], The inclusion complex of cucurbituril with fluorouracil treatment in KB cells showed higher anticancer potential than fluorouracil treatment in KB cells when compared to the control. The IC<sub>50</sub> value of 5-fluorouracil was found to be higher compared to the inclusion complex of cucurbituril with fluorouracil. The interesting results obtained, might open new avenues for the use of CB[6] host-guest complexes in anticancer and drug delivery studies.

**Keywords:** cucurbituril[6]; fluorouracil, supramolecular chemotherapy; host-guest complex.

## 1. INTRODUCTION

Supramolecular chemistry is defined as “chemistry beyond the molecule” that aims at designing and implementing functional chemical systems based on molecular components held together by noncovalent intermolecular forces. It has grown into a major field and has stimulated numerous developments at the interfaces with biology and physics, from ba-

sic knowledge to applications, from noncovalent interactions to drug design, and from materials and polymers to solid state engineering [1]. Supramolecular chemistry is a promising field for the development of new pharmaceutical therapies by understanding the interactions at a drug binding site. The area of drug delivery has also made critical advances as a result of supramolecular chemistry providing encapsulation and targeted release mechanisms. In addition, supramolecular systems are designed to disrupt protein-protein interactions that are important to cellular function [2 – 5].

Self-assembled materials can be of great utility in controlling drug delivery processes (actively or passively) and

<sup>1</sup> Department of Chemistry, Madras Christian College (Autonomous), East Tambaram, Chennai, 600059 India.

<sup>2</sup> Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar, Tamil Nadu, 608002 India.

\* e-mail: jabapriyat@gmail.com

enabling the growth and regeneration of cellular tissue (*ex vivo* or *in vivo*) [6].

Conventional chemotherapy has drawbacks ranging from a poor solubility/stability of drugs in physiological environments to their limited efficacy, drug resistance and severe treatment-related side effects of drugs to healthy tissues greatly limiting clinical applications [7]. Supramolecular chemotherapy integrating non-covalent interactions and traditional chemotherapy is highly hopeful and can be aptly used for targeted drug delivery. By taking advantage of supramolecular chemistry, the limitations of traditional chemotherapy for clinical applications can be untangled effectively [7, 8].

Macrocyclic molecules such as crown ethers, cyclodextrins, calixarenes, cucurbiturils and pillararenes, usually have hydrophobic cavities in which the guests can be encapsulated [7, 9, 10]. These magnificent macrocycles provide ideal platforms for the fabrication of supramolecular chemotherapeutic agents through host–guest molecular recognition. The solubility/stability of poorly soluble anticancer drugs can be significantly improved in physiological environments upon the formation of host–guest complexes. High accumulation of an anticancer drug in tumor can be achieved using supramolecular self-assembly, remarkably enhancing the efficacy of the supramolecular chemotherapeutic agents and reducing the side effects towards normal tissues [11, 12]. The dynamic nature of non-covalent interactions makes supramolecular chemotherapy more versatile than traditional chemotherapy and nanomedicines that have a shortage of stimuli-responsiveness [7].

Platinum-based anticancer chemotherapy is coupled with severe side effects and multidrug resistance [7]. Wheate and co-workers [13] encapsulated cisplatin in CB[7] to achieve enhanced anticancer efficacy towards human ovarian carcinoma cell lines. *In-vivo* study revealed that the CB[7]\*cisplatin complex could be used for the treatment of drug resistant human cancer [14]

Zhang and co-workers used the clinical antitumor drug oxanthazole (OX) to form a host–guest complex with CB[7]. The cytotoxicity of OX to the normal colorectal cells can be significantly decreased by formation of the CB[7]\*OX complex. More importantly, CB[7]\*OX exhibited higher antitumor activity than OX itself, because the release of OX from CB[7]\*OX and simultaneous consumption of spermine by CB[7] resulted in cooperatively enhanced anticancer performance [14].

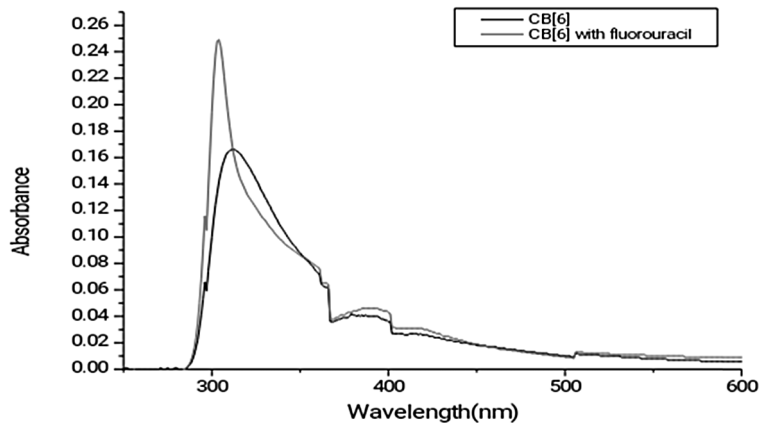
Supramolecular drugs could potentially lead to increase in the drug tendency to cross the blood–brain barrier and to be absorbed in the cells by fully taking advantage of host–guest chemistry, thereby enhancing anticancer efficacy [7, 15–18]. *In vitro* cell growth assays using free cucurbiturils and some linear cucurbituril derivatives showed no cytotoxicity at up to millimolar concentrations [19]. These spectacular anticancer studies on CB[n]s kindle the interest of developing new efficient alternative chemopreventive therapies with minimal adverse effects.

Cucurbiturils have been shown to form host-guest complexes for a wide range of organic and inorganic small molecule drugs, where encapsulation is facilitated through hydrophobic effects within the cucurbituril cavity and further stabilized by hydrogen bonding or ion-dipole interactions within cucurbituril portal [20–22]. Encapsulation of drugs within the homologs of CB[6], CB[7] or CB[8] can impart enhanced chemical and physical stability, improve drug solubility and control drug release. Cucurbiturils have been included in tablets for oral delivery and inserts for nasal delivery [23]. Hydrophobic drug molecules can easily get assembled in the cavity of cucurbiturils through noncovalent interactions. The physicochemical properties and structural aspects of the host-guest complexes alter and lead to enhancement in the solubility and bioavailability of drugs. In the present work, the ability of host-guest complexes obtained by the inclusion of anti-cancer drug fluorouracil in the host supramolecule of cucurbiturils so as to inhibit the generation of reactive oxygen species (ROS) has been studied.

Reactive oxygen species (ROS) are chemically reactive oxygen radicals and non-radical derivatives of oxygen. ROS play a role in cell signaling, including the apoptosis and gene expression. ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals may function in cell signaling processes at both low and higher concentrations. These species can damage cellular macromolecules and participate in apoptosis. Previous studies reported that the ROS production can be measured by chemiluminescence (CL) with chloramphenicol (CP) in cyclodextrin (CD) complex [24]. The intracellular ROS generation ability of biotin conjugated toluidine blue (TB-B) and 2TB-B@CB[8] were examined by DCFH-DA [25, 26]. Investigation of the CB[7] conjugated to a triglycosylated tetraphenyl porphyrin (TPP-3Man-CB[7]) revealed its effect on the growth kinetics of Gram-negative bacteria of *Escherichia coli* (*E. coli*) [27]. The singlet oxygen generation efficiency of porphyrins is readily modified with four positive charges (TPOR) enhanced in the presence of CB[7] [28, 29]. Naphthalene–methylpyridinium attached porphyrin (TPOR) inclusion complex with CB[7] in water to be utilized as a photosensitizer for antibacterial treatment was studied by Liu, et al. [28]. The antibacterial activities of TPOR and TPOR/CB[7] toward *E. coli* demonstrated the ability of CB[8] ternary complex with this photosensitizer and the residue of protein to produce oxidative photocleavage of proteins [30].

This paper focuses on the studies of structural ROS generation in the CB inclusion complex with fluorouracil (anticancer drug) and of host-guest complex. In the present work, effort was made to synthesize the inclusion complex of anticancer drug 5-fluorouracil as guest with CB[6], and its structure was characterized using UV-Vis spectroscopy, TG-DTA and XRD techniques.

5-Fluorouracil (FLU) is widely used for the treatment of a number of cancers, including colorectal and breast cancers, and cancers of the aero digestive tract. Although FLU in combination with other chemotherapeutic agents improves



**Fig. 1.** UV-Vis spectra of CB[6] and host-guest complex of CB[6] with fluorouracil.

response rates and survival in breast, head and neck cancers, it is in colorectal cancer that FLU has the greatest impact [31].

The structural and ROS studies of the following samples were studied 1) 5-fluorouracil, 2) inclusion complex of cucurbituril with fluorouracil,

The structural study of CB[6] complex with 5-fluorouracil was carried out using UV-Vis, TG, DTG and DSC, and XRD techniques.

## 2. EXPERIMENTAL PART

### 2.1. Preparation of Host-Guest Complexes of CB[6] with 5-Fluorouracil

Equimolar solutions of CB[6] and 5-fluorouracil were mixed in the same ratio (1:1) with aqueous HCl solution as the solvent. The final clear solution was heated above room temperature, and the product obtained was filtered off, dried, and the target complex was obtained as a powder.

This synthesised CB[6] and 5-fluorouracil host-guest complex was used for studies of its structure, antibacterial activity, cytotoxicity and ROS generation.

### 2.2. Structural Study of CB[6] Host-Guest Complexes with 5-Fluorouracil

**UV-Vis spectroscopy of CB[6] complex with 5-fluorouracil.** The interaction of CB[6] and the guest fluorouracil were analysed using electronic absorption spectra. The UV spectra were recorded for free CB[6] and inclusion complex of CB[6] with fluorouracil at 0.025M concentration in aqueous solution (Fig. 1). The UV-Vis spectrum of the inclusion complex exhibited maximum absorbance with a blue shift at 302 nm whereas the maximum absorbance for free CB[6] was observed at 318 nm, this notable shift in wavelength proves the formation of inclusion complex of CB[6] with fluorouracil. Earlier studies of inclusion complexes of cucurbituril also show similar results [17–20].

**Thermal studies of CB[6] complex with 5-fluorouracil.** TGA of host-guest complex of fluorouracil with CB[6] (Fig. 2) shows a gradual weight loss of -9.20% from ambient temperature 105.8°C up to 240°C that is assigned to the removal of water. The onset temperature of CB[6] decomposition for host-guest complex shows almost 40°C difference, and the initial weight loss changes shows a vast difference and the DTG maximum of the complex is shifted toward lower temperature (200°C). The first inflection is observed at 240°C and the second inflection is obtained at 480°C. These significant changes observed in thermal studies of the host-guest complex and parent CB[6] confirm the formation of a host-guest complex. Complete weight loss is not observed in the TGA curves of the host-guest complex, the residual mass at 1099°C is 13.72%. Earlier thermal studies of CB[7] host-guest complexes also gave similar results [32]. The DSC curve of CB[6] shows an endothermic peak at 514.9°C with a heat flow of -4.24 mW/mg and the peak covers an area of 491.8 J/g. The host-guest complex of fluorouracil with CB[6] gives a sharp endothermic peaks at 403.3°C with a heat flow of -0.5 mW/mg and the peak covers partially an area of 528.2 J/g. Large variations observed in DSC curves of the complex compared with free host CB[6] also reaffirm the formation of stable host-guest complexes.

**Powder XRD of CB[6] complex with 5-fluorouracil.** The powder XRD of host-guest complex of cucurbit[6]uril and fluorouracil (Fig. 3a) shows multiple peaks and sharp peaks are observed at  $2\theta = 10^\circ, 15^\circ, 23^\circ, 25^\circ$  and  $29^\circ$ . Multiple peaks in the graph of intensity vs.  $2\theta$  values indicate that crystalline structure is maintained in the samples. X-ray powder diffraction patterns show numerous reflections in low-angle regions indicative of crystalline phase. The XRD pattern of host-guest complex is different from that of parent compound (Fig. 3b), which confirms the formation of host-guest complexes [33, 34].

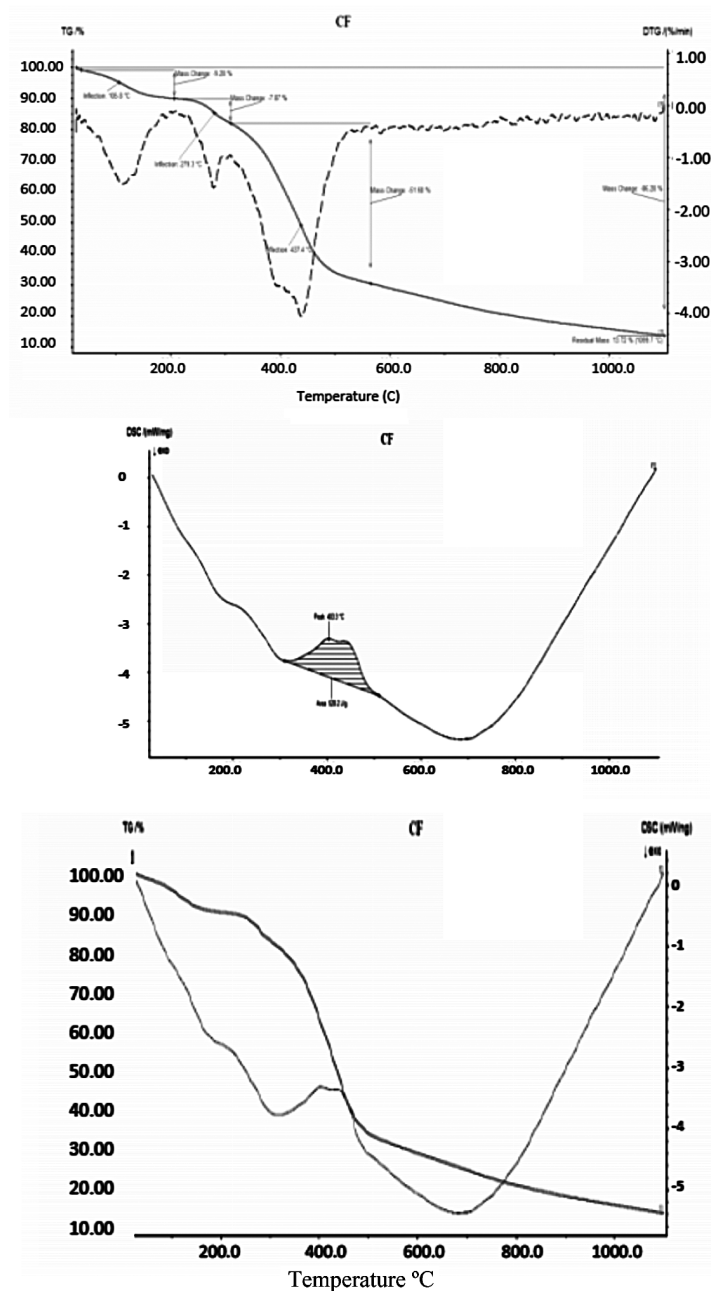


Fig. 2. TG, DTG and DSC curves of CB[6] complex with 5-fluorouracil.

### 2.3. Effect of Fluorouracil and Host-Guest Complex of Cucurbituril with Fluorouracil on the Cytotoxicity by MTT Assay in KB Cells

To assess the nontoxic concentrations of fluorouracil (FLU) and host-guest complex of cucurbituril (CB) with fluorouracil in cytotoxic studies, MTT based cytotoxicity assay in KB cells were used.

**Materials and methodology.** KB cells were purchased from NCCS (Pune). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), glutamine, penicil-

lin-streptomycin and trypsin neutralizer solution were purchased from Himedia (India), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), 2,7-diacetyl dichlorofluorescein (DCFH-DA), rhodamine 123, ethidium bromide and acridine orange were purchased from Sigma Co. (St. Louis, USA). All other reagents used were of analytical grade; these reagents and solvents were obtained from S. D. Fine Chemical, Mumbai and Fisher Inorganic and Aromatic Ltd. (Chennai).

Cultured KB cells ( $1 \times 10^5$  cells/mL) were taken in a 96 well plate. Then the cells were treated with different concen-

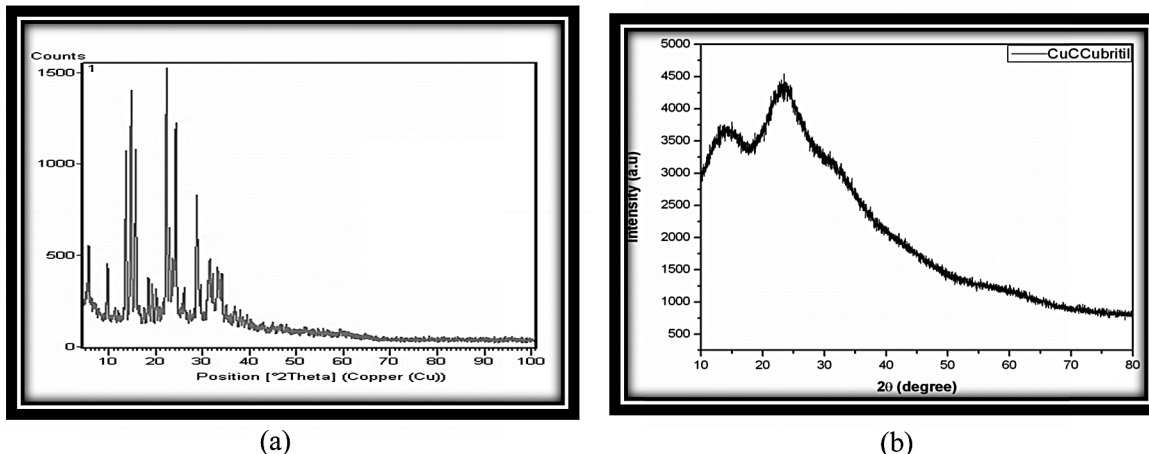


Fig. 3. Powder XRD pattern of (a) inclusion complex of CB[6] with fluorouracil and (b) host CB[6].

tration of FLU, CB-FLU (0.9 – 250 µg). Then, the cells were incubated in 5% CO<sub>2</sub> and 95% O<sub>2</sub> environment at 37°C for 24 h, MTT (0.5 mg/mL) was added to the cells, and these were further incubated for another 4 h. Finally, the cells were centrifuged for 10 min and the supernatant was removed, and 200 µL DMSO was added to each tubs. The absorbance was measured in a microplate reader at 540 nm and images were captured under microscope [35 – 37]. The percentage cytotoxicity was calculated by the following formula:

$$\%Viability = \frac{\text{Total number of viable cell}}{\text{Total number of viable andnonviable cells}} \times 100.$$

**Effect of 5-flurouracil and host-guest complex of CB[6] with 5-flurouracil on cytotoxicity by MTT assay.** Earlier studies of the cytotoxicity of CB based compounds showed interesting results. Kim and coworkers demonstrated the non-toxicity of CB molecules with ED<sub>50</sub> levels of more than 100 µM against human lung and ovarian cancer cells

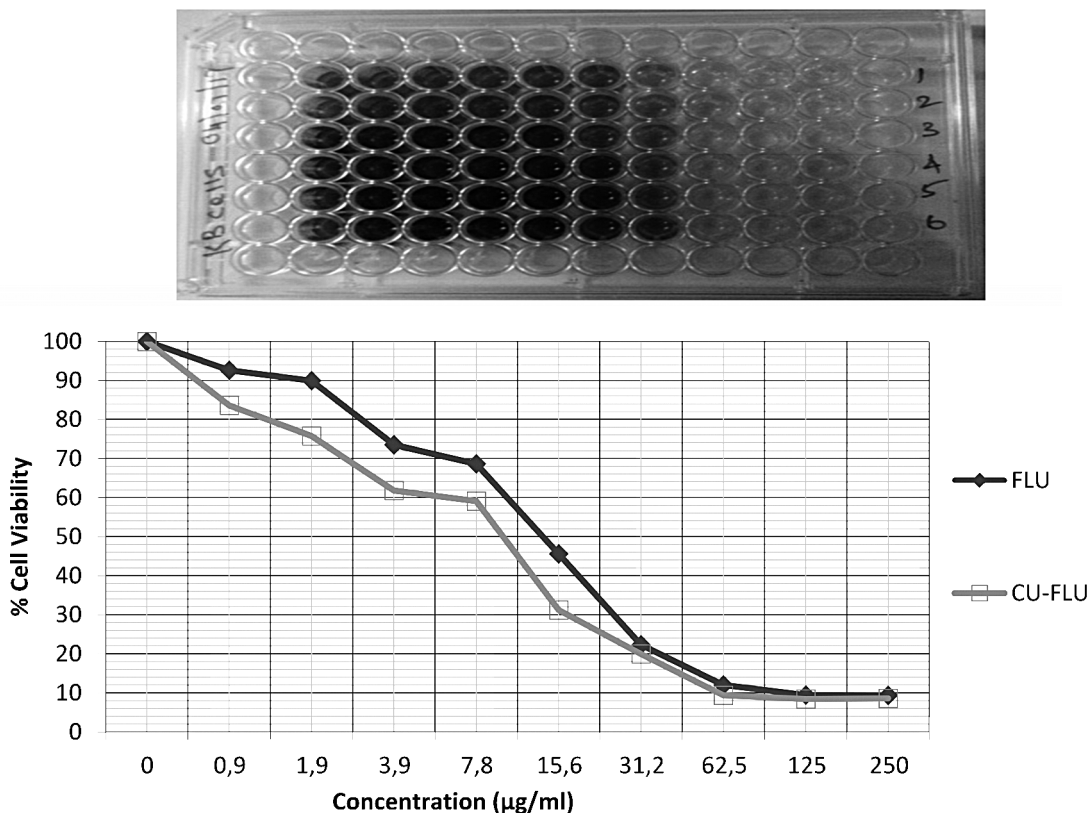


Fig. 4. (I) Cultured KB cells treated with various concentration of host-guest complexes in microplate reader; (II) effect of FLU and CB-FLU on cytotoxicity by MTT assay in KB cells. Cell viability values are given as mean ± SD % for Flu and CB-Flu studied at 10 concentrations.

**TABLE 1.** T-Test Analysis of Cytotoxicity Data for Flu and CB-Flu Independent Samples

Comparison of Flu and CB-Flu	T-test for Equality of Means				
	T	Df	Sig. (2-tailed) P value	95% Confidence interval of differences	
				Lower	Upper
	.410	18	0.687	-27.011	40.115

(Jeon, et al., 2005). *In vitro* cell viability testing of CB7 by MTT assay in CHO-K1 cells showed no significant cytotoxicity at up to 1 mM concentration and 3 h incubation time, while after 48 h incubation, an  $IC_{50}$  value of 0.53 mM was determined (Uzunova, et al., 2010).

In the present work, the cytotoxic effect of 5-fluorouracil (FLU), host-guest complex of CB[6] with 5-fluorouracil (CB-FLU) and other host guest complexes has been determined based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity [35]. This is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate hydrogenase or mitochondrial reductase. MTT enters the cells and passes into mitochondria, where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with isopropanol in an organic solvent and the released formazan reagent is measured spectrophotometrically. Since the MTT reduction can only occur in metabolically active cells, the level of activity is a measure of viability of the cells [36, 37]. Figure 4 shows the cytotoxic effect of FLU and CB complex with FLU as determined by MTT assay. The cells were treated with various concentrations of FLU and CB-FLU (0.9 – 250  $\mu$ g) for 24 h incubation, which revealed a dose-dependent inhibition of cell proliferation. Maximum cell death was observed at 250  $\mu$ g concentration. From these results, CB-FLU showed maximum activity in KB cells as compared to other host-guest complexes. The CB-FLU doses of 2.5, 5, and 10  $\mu$ g were selected for further studies. Note that free CB[6] cytotoxicity was not used for the study because of its sparingly soluble nature, whereas CB-Flu solubility increased and could be used for further studies.

**Statistical analysis.** All results were expressed as mean of six ( $n = 6$ ) determinations. The data were statistically analyzed using one-way analysis of variance (ANOVA) on SPSS (statistical package for social sciences) and the group means were compared by Duncan's Multiple Range Test (DMRT).

#### 2.4. Statistically Processed Data on Cytotoxicity of Flu and CB-Flu with Indication of Confidence Intervals

Analysis of the T-test results gave the P values (Table 1) and frequency statistics (Table 2). *In vitro* cell viability testing of the host-guest complex of CB[6] with anticancer drug flurouracil by MTT assay in KB cells showed no significant

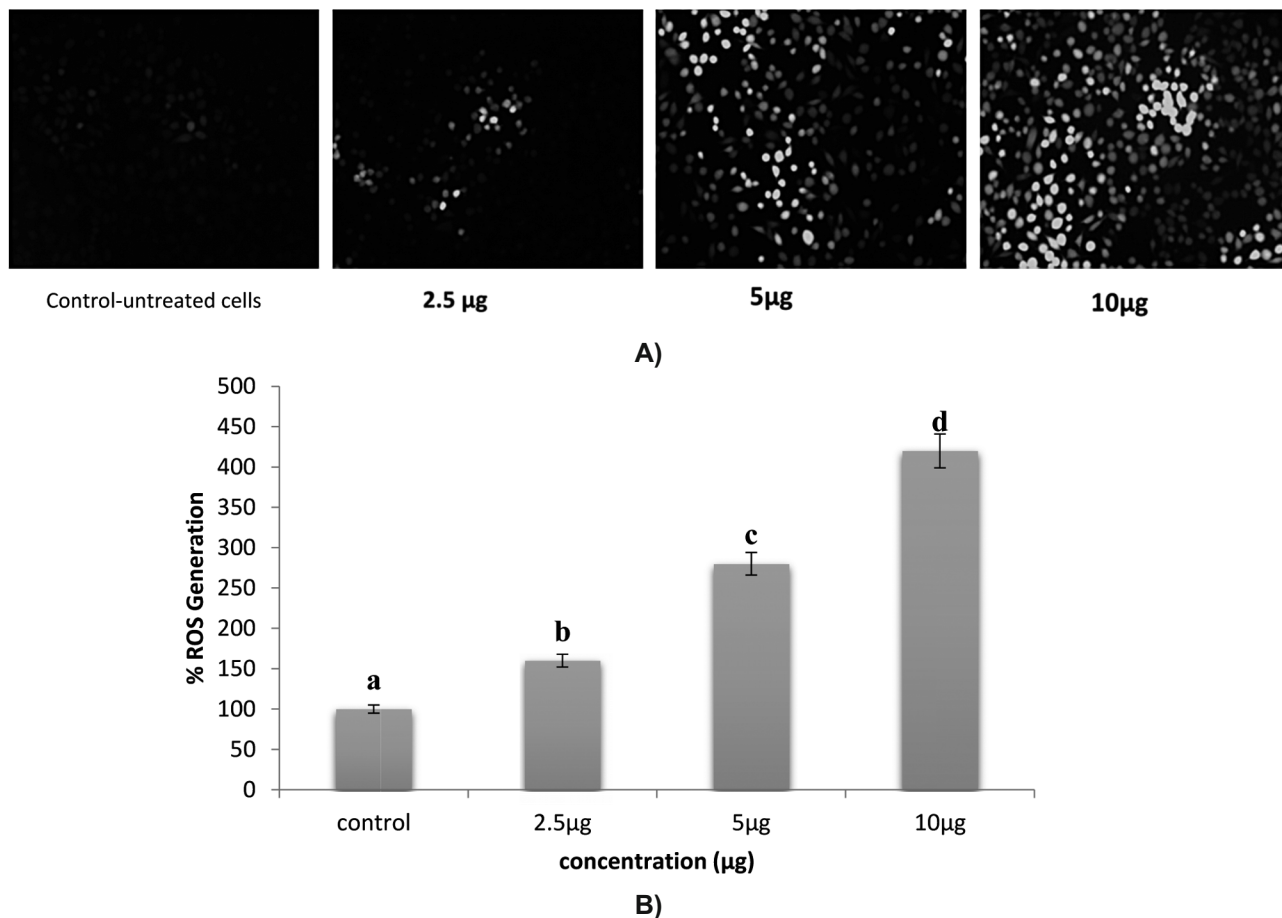
cytotoxicity and the maximum cell death was observed at 250  $\mu$ g dose after 24 h incubation time. Increasing CB-FLU (2.5, 5, 10  $\mu$ g) doses were selected for further studies. Owing to low solubility of the CB-FLU host-guest complex, precise determination of its toxicity level was difficult. From earlier works, it was known that CB[5], CB[7], and several acyclic CB containers were also tested for their toxicity and bioactivity and found to be non-toxic within the concentration range of interest. Data on the cell viability for Flu and CB-Flu at 10 concentrations have been analyzed and the statistical details are given in Table 2.

#### 2.5. Effect of Fluorouracil and Host-Guest Complex of Cucurbituril with Fluorouracil on Intracellular ROS Generation

Reactive oxygen species are biological molecules which play significant roles in cardiovascular physiology and contribute to disease initiation, progression, and severity. Excessive ROS accumulation will cause oxidative stress and damage to cells. [38, 39]. Previous studies reported that ROS production measured by chemiluminescence (CL) with chloramphenicol (CP). The CP:CD complex showed a rise in ROS[24]. ROS generation ability of TB-B and 2TB-B@CB[8], the generated ROS could be detected after light irradiation, the cells exhibited similar green fluorescence[25, 26]. Investigation of TPP-3Man-CB7, upon irradiation of *E. coli* suspension at higher concentration TPP-3Man-CB7 gives 100%, while in the dark it is only

**TABLE 2.** Cell Viability Effect of Flu and CB-Flu at 1° Concentrations (Statistical Details–requeencies –Statistics)

Frequencies		Flu	CB-Flu
N	Valid	10	10
	Missing	0	0
Std. Error of Mean		11.683	10.896
Std. Deviation		36.943	34.457
Skewness		-.029	.254
Std. Error of Skewness		.687	.687
Percentiles	25	11.37	9.17
	50	57.08	45.16
	75	90.60	77.70



**Fig. 5.** Effect of CB-FLU complex on intracellular ROS generation using DCFH-DA (2'-7'- dichlorofluorescein diacetate) in KB cells (keratin-forming tumor cell line).

around 10% [27]. The antibacterial activities of TPOR and TPOR/CB7 toward *E. coli* were investigated and TPOR/CB7 was found to be very efficient photosensitizer for antibacterial photodynamic therapy [28, 29]. The ternary complex of CB[8] with photosensitizer upon light irradiation generated singlet oxygen capable of damaging proteins that could regulate the cell signalling process and apoptosis of cells [30].

The complexation of diphenyleneiodonium, a bioactive halonium ion, with CB[7] and CB[8] has been recently studied by Yin and R. Wang [40]. The host-guest binding experiments revealed 1:1 complexation stoichiometry with CB[7] ( $K_a = 3 \times 10^4 \text{ M}^{-1}$ ) and 1:2 complexation with CB[8] ( $K_a = 2 \times 10^{12} \text{ M}^{-1}$ ). Excitingly, the complexation was shown to modulate the inhibitory activity of diphenyleneiodonium against ROS generation and to improve its cardiotoxicity [40, 41].

Cucurbit[6]uril (CB[6]) acts as a host and encapsulates [5]-rotaxane ring inside. Further studies revealed that it has ability to generate reactive oxygen species (ROS) including singlet and the complex remains stable at physiological pH (7.4) for prolonged times [42].

Thus, the earlier anticancer studies have proved that the supramolecular host-guest complexes can serve as an anticancer agent.

#### 2.6. Measurement of Intracellular ROS Generation

In this work, the intracellular ROS generation was measured using DCFH-DA staining. Dichlorodihydrofluorescein diacetate (DCFH-DA) is the cell permeable fluorescent and chemiluminescent probe used for direct measurement of the intracellular ROS levels. DCFH-DA is a nonpolar dye, converted into the polar derivative DCFH by cellular esterases that are nonfluorescent but switched to highly fluorescent DCF when oxidized by intracellular ROS and other peroxides [43, 44].

Accumulation of DCF in cells may be measured by an increase in fluorescence at 530 nm when the sample is excited at 485 nm. The results were expressed as percentage fluorescence intensities. In addition, the fluorescence cells were analyzed using a phase contrast fluorescence microscope with blue filter (460 nm). Phosphate buffered saline (PBS) and 2'-7'-diacetyl dichlorofluorescein diacetate (DCFH-DA) were used as reagents.

**Methodology.** The percentage of ROS levels was estimated in the control and treated KB cells. Cells were seeded ( $1 \times 10^6$  cells/well) in 6-well plate treated with CB-FLU at different concentration and kept in a CO<sub>2</sub> incubator for 24 h. After 24 h incubation 1 mL of cells were incubated with 100 mL DCFH-DA for 10 min at 37°C. Finally, cells were washed thrice with PBS and the fluorescence intensity was recorded using spectrofluorometer and the images were captured using fluorescence microscope (460 nm). Photomicrographic image (Fig. 5a) and spectrofluorometric readings (Fig. 5b) of the DCF fluorescence of the control and CB-FLU treated KB cells are shown. Untreated KB cells show weak fluorescence DCF. CB-FLU (2.5, 5, 10 µg) treated KB cell shows increased ROS generation indicates deep DCF fluorescence intensity. The images were acquired by floid cell imaging station. According to spectrofluorometric readings quantitative values are given as the mean (SD) of six experiments in each group (values not sharing a common superscript differ significantly at  $P < 0.05$  DMRT)).

#### 2.7. Effect of CB-FLU on Intracellular ROS Generation Studied by DCFH-DA Staining in KB Cells

Photomicrographic images and spectrofluorometric readings (Fig. 5) showed potent anti-proliferation at submicromolar concentrations after 24-h treatment. The images were acquired by floid cell imaging station. CB-Flu host guest complex consistently exhibited much lower IC<sub>50</sub> values in the tested cancer KB cell lines. The IC<sub>50</sub> value of 5-fluorouracil and inclusion complex of cucurbituril with fluorouracil was found to be 13 µg/mL and 10 µg/mL, respectively. The number of cells showing features of ROS was counted as a function of the total number of cells present in the field. After 24 h incubation 1 mL of cells were incubated with 100 mL DCFH-DA for 10 min at 37°C. Accumulation of DCF in cells is measured by an increase in fluorescence at 530 nm when the sample is excited at 485 nm. The results were expressed as percentage fluorescence intensities, the CB-Flu intensities were compared with the control considered as 100%. The intracellular ROS generation was measured using DCFH-DA staining. Figure 5B depicts the levels of ROS generation in control and CB-FLU treated cells. KB cells were treated with various concentration of CB-FLU (2.5, 5, 10 µg) which showed significantly increased levels of ROS generation. Treated cells produces intense green fluorescence compared to untreated control cells. Untreated KB cells show weak fluorescence DCF. CB-FLU (2.5, 5, 10 µg) treated KB cell shows increased ROS generation indicate deep DCF fluorescence intensity.

(A) Photomicrographic images of the control and CB-FLU treated KB cells. Untreated KB cells show weak DCF fluorescence. CU-FLU (2.5, 5, 10 µg) treated KB cells show increased ROS generation as indicated by deep DCF fluorescence intensity. The images were acquired by floid cell imaging station; (B) spectrofluorometric readings of

DCF (2',7'-dichlorofluorescein) fluorescence in control and CB-FLU treated cells.

### 3. CONCLUSION

In this study, host-guest complexes of CB[6] with anticancer drug 5-fluorouracil were synthesized and their structure, cytotoxicity and ROS generation characteristics were studied. The UV, thermal and XRD studies confirmed the formation of host-guest complex and it was found that the cytotoxicity of anticancer drug against cancer cells significantly increased due to fluorouracil encapsulation into the hydrophobic cavity of CB[6]. The inclusion complex of cucurbituril with fluorouracil used for the treatment in KB cells showed higher anticancer potential than that of fluorouracil treatment in KB cells when compared to the control. The IC<sub>50</sub> values of 5-fluorouracil and the inclusion complex of cucurbituril with fluorouracil were found to be 13 and 10 µg/mL, respectively. The cytotoxicity of anticancer drugs to cancer cells was significantly increased by encapsulating 5-fluorouracil into the hydrophobic cavity of CB[6] and delivering them only at cancer cells which have lower pH compared to normal cells. The host-guest complex exhibits significant cytotoxic activity, the more so considering the high association constants that the host-guest complexes based on CBs can have and that CBs have no intrinsic cytotoxicity in many human cancer lines [45].

Previously reported studies provided convincing evidence that the fluorescence images of CB host-guest complexes of basic dyes can cross the cell membrane. These complexes displayed *in vitro* compatibility with human kidney and liver cells, demonstrated no ill effects from high dose *in vivo* studies on mice, as well as an increased potency of some anti-cancer drugs when first encapsulated in the hosts [46].

Thus, it is very likely that other CB host-guest complexes will behave similarly. The present work showed potent anti-proliferation at submicromolar concentrations changes in the CB-FLU host-guest complexes with KB cells in a concentration dependent manner. Considering the interesting results obtained, our findings might open new avenues for the use of CB[6] host-guest complexes in drug discovery.

### ACKNOWLEDGMENTS

We are grateful to our esteemed Institution of Madras Christian College, Chennai, India. We thank the Principal, MCC, Head, Teaching and Non-teaching Staff of the Department of Chemistry, MCC and UGC, FDP program, India. We thank our Family and Friends and above all The Lord Almighty for everything.

### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.



## REFERENCES

1. M. L. Jean, *Chem. Soc. Rev.*, **46**, 2378 – 2379 (2017).
2. R. B. Silverman, *The Organic Chemistry of Drug Design and Drug Action*, 2nd Ed., Elsevier Academic Press: London (2004), pp. 29 – 32.
3. B. Krishnamoorthy, M. Muthukumran, and G. Sumithira, *Int. J. Adv. Pharm. Gen. Res.*, **3** (1), 37 (2015).
4. M. Zhang, D. Xu, X. Yan, J. Chen, et al., *Angew. Chem. Int. Ed.*, **51**, 7011 – 7015 (2012).
5. B. A. David, K. S. David, and W. S. Jonathan, *Chem. Soc. Rev.*, **46**, 2404 – 2420 (2017).
6. V. M. Tysseling, V. Sahni, E. T. Pashuck, et al., *J. Neurosci. Res.*, **88**, 3161 – 3170 (2010).
7. Z. Jiong, Y. Guocan, and H. Feihe, *Chem. Soc. Rev.*, **46**, 7021 – 7053 (2017).
8. J. Li and X. J. Loh, *Adv. Drug Delivery Rev.*, **60**, 1000 – 1017 (2008).
9. G. Yu, K. Jie, and F. Huang, *Chem. Rev.*, **115**, 7240 – 7303 (2015).
10. Z. Niu, F. Huang, and H. W. Gibson, *J. Am. Chem. Soc.*, **133**, 2836 – 2839 (2011).
11. R. Haag, *Angew. Chem. Int. Ed.*, **43**, 278 – 282 (2004).
12. H. J. Yoon and W. D. Jang, *J. Mater. Chem.*, **20**, 211 – 222 (2010).
13. J. A. Plumb, B. Venugopal, R. Oun, et al., *Metallomics*, **4**, 561 – 567 (2012).
14. Y. Chen, Z. Huang, H. Zhao, et al., *ACS Appl. Mater. Interface.*, **9**, 8602 – 8608 (2017).
15. E. A. Appel, M. J. Rowland, X. J. Loh, et al., *Chem. Commun.*, **48**, 9843 – 9845 (2012).
16. X. J. Loh, *Mater. Horiz.*, **1**, 185 – 195 (2014).
17. P. Xing and Y. Zhao, *Adv. Mater.*, **28**, 7304 – 7339 (2016).
18. M. Eric, L. Xiaoxi, J. Roymon, et al., *RSC Adv.*, **2**, 1213 – 1247 (2012).
19. W. Shonagh, O. Rabbab, J. M. Fiona, et al., *Isr. J. Chem.*, **51**, 616 – 624 (2011).
20. G. Hettiarachchi, D. Nguyen, J. Wu, et al., *PLoS One*, **5**(5), e10514 (2010).
21. F. Xing, J. L. Xiao, et al., *Inorg. Chem. Commun.*, **12**, 844 – 849 (2009).
22. S. Walker, R. Oun, F. J. McInnes, et al., *Isr. J. Chem.*, **51**, 616 – 624 (2011).
23. L. Jason, M. Pritam, C. Sriparna, et al., *Angew. Chem. Int. Ed.*, **44**, 4844 – 4870 (2005).
24. L. Lu, S. Zhu, H. Zhang et al, *RSC Adv.*, **5**, 14114 – 14122 (2015).
25. B. A. Lindig, M. A. J. Rodgers, A. P. Schaap. *J. Am. Chem. Soc.*, **102**, 5590 – 5593 (1980).
26. K. Han, Q. Lei, S. B. Wang, et al., *Adv. Funct. Mater.*, **25**, 2961 – 2971 (2015).
27. M. Ozkana, Y. M. Kesera, A. Kocb, et al., *J. Porphyrins Phthalocyanines*, **23**, 1406 – 1413 (2019).
28. F. Liu, A. S. Y. Ni, Y. J. Lim, et al., *Bioconjugate Chem.*, **23**, 1639 (2012).
29. L. Chen, H. Bai, J. F. Xu, et al., *ACS Appl. Mater. Interfaces.*, **9**, 13950 – 13957 (2017).
30. W. Lei, G. Jiang, Q. Zhou, et al., *Phys. Chem. Chem. Phys.*, **12**, 13255 – 13260 (2010).
31. B. L. Daniel, D. P. Harkin, and G. J. Patrick, *Nat. Rev. Cancer.*, **3**, 330 – 338 (2003).
32. P. Germain, J. M. Letoffe, M. P. Merlin, and H. J. Buschmann, *Thermochim. Acta.*, **315**, 87 – 92 (1998).
33. A. C. Gomes, C. I. Magalhães, T. S. Oliveira, et al., *Dalton Trans.*, **45**(42), 17042 – 17052 (2016).
34. P. Subramanian, S. Mohamed, and Y. Alias, *Int. J. Mol. Sci.*, **11**, 367 (2010).
35. T. Mosmann, *J. Immunol Methods*, **65**, 55 (1983).
36. <https://www.sciencedirect.com/topics/neuroscience/mtt-assay> (last accessed June 2021)
37. <https://www.intechopen.com/books/genotoxicity-a-predictable-risk-to-our-actual-world/in-vitro-cytotoxicity-and-cell-viability-assays-principles-advantages-and-disadvantages> (last accessed July 2021)
38. J. Wang, B. Luo, X. Li, et al., *Cell Death Dis.* **8**(6), e2887 (2017).
39. K. K. Griendling, R. M. Touyz, J. L. Zweier, et al., *Circ. Res.*, **119**(5), e39–e75 (2016).
40. H. Yin and R. Wang, *Israel J. Chem.* **58**, 188 – 198 (2018).
41. D. Das, K. I. Assaf, and W. M. Nau, *Front. Chem.*, **7**, 619 (2019)
42. <http://repository.bilkent.edu.tr/bitstream/handle/11693/54021/10355041.pdf?sequence=1&isAllowed=y> (last accessed Aug 2021)
43. R. P. Rastogi, S. P. Singh, D.-P. Hader, et al., *Biochem. Biophys. Res. Commun.*, **397**(3), 603 – 607 (2010).
44. P. L. Bustos, A. E. Perrone, N. Milduberger, et al., *Parasitology*, **142**(8), 1024 – 1032 (2015)
45. P. M. Navajas and H. Garcia, *J. Photochem. Photobiol. A*, **204**, 97 – 101 (2009).
46. V. K. Igor and V. A. Eric, *Chem. Soc. Rev.*, **46**, 2385 – 2390 (2017).