Anticancer Activity of New Copper (II) Complexes with 6-Thiguanine Drug

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Abstract: A new complex has been synthesized of Cu (II) complex with 6-thioguanine and phyico-chemical characterized by amperometry, polarography elemental analysis and FTIR spectroscopy. After Synthesis of metal complex, it was evaluated it for antibacterial and antifungal activities against various pathogenic microorganisms such as; *Streptococcus aureus, Proteus. M.*, *klebsiella pneumonia and Asperginus niger, Nigrosporan S.P*. B16-F10 melanoma cell and C-57BL/6 mice has been used for anticancer screening of metal complex for *in vitro* and *in vivo* study. The result of pharmacological studies with M: L revealed that the complex is more potent as compared to the pure drug as regards to its anticancer activity.

Keywords: Cu (II) Complex, Polarography, Amperometry, Biological investigation, anticancer activity.

1. INTRODUCTION

6-thioguanine, 2-amino-7H Purina -6-thiol has been used in treatment of various type of tumors, it is well known that thiopurines inhibit the synthesis of DNA and RNA and have been used successfully in the treatment of acute leukemia [1, 2]. Organo-metallic compounds have been used in medicine for centuries. Metal play essential role in pharmaceutical industry. The metallo-elements present in trace quantities play vital role at the molecular level in the system. Copper as a component of numerous enzymes is involved in energy production, is necessary for neurotransmission in the brain and is active in cell protection from the damage generated by the free radicals. The copper deficiency is associated with the anemia and bone demineralization. Copper is most abundant and essential metal in our body system [3].

Cu(II)- based complexes appear to very promising candidates for anticancer therapy, an idea supported by a considerable number of researchers [4-7] describing the synthesis and cytotoxic activities of numerous Cu(II) complexes and copper (II) complexes conta-ining semicarbazones have also displayed biological properties [8-10]. The study of copper complex of anticancer drug 6-thiguanine have carried out by phyico-chemically microbially and pharmacologically. The metal ligand complexation equilibrium have been studied and elemental and IR spectral analysis has been worked out which given probable formula for complex is to 1:1. Various pathogenic bacteria like *Streptococcus aureus, Prosteus M.*, *klebsiella pneumonia* and fungal strains such as *Asperginus niger, Nigrosporan S.P* have been applied for microbial study using disc diffusion method. B16-F10 melanoma cell and C-57BL/6 mice were used for the *in vitro* and *in vivo* anticancer study of complex compound, respectively. The results of physiochemical method, microbial and pharmacological studies with Cu (II) 6-thioguanine complex may be suggested to the therapeutic experts for its possible use as more effective anticancer drug.

2. MATERIALS AND METHODS

All the chemical used were of analytical grade, the drug 6-thioguanine was procured form Sigma Chemical Company, USA. Standard solution of Cu (II) 2 mM 6-thioguanine 2mM and Ammonium Buffer 0.1M solution 5% of 95% ethyl alcohol prepared, Polarographic / voltammetric measurement was carried out using a ion analyzer, Model 797A Computrace Metrohm, Herisau, Switzerland with stand three electrodes containing a DME dropping mercury electrode as a working electrode, a coiled platinum wire as an auxiliary electrode and saturated calomel electrode (SCE) as a reference electrode.

2.1. Electrochemical Studies of Cu(II)- 6-Thioguanine Complex

For the study of metal: ligand (M:L) complexation equilibrium experiment sets were prepared by keeping

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overall Cu(II) and Ammonium Buffer (supporting electrolyte) concentration fixed at 2 mM and 0.1M, respectively. The ligand concentration varied from 0.0 to 15mM. The pH of the test solution was adjusted to 10.4 ±0.02 using HCl/NaOH solution. The test solutions were deaerated by bubbling nitrogen gas for 15min before recording the polarogram. The amperometeric titrations were performed on a manually operated set up equipped with a polyflex galvanometer (sensitivity 8.1 × 10^{-9} amp per div) and an ajco vernier potentiometer. The capillary characteristics of DME had $m^{2/3} t^{1/6}$ value of 2.5 $mg^{2/3} S^{-1/2}$ at 50 cm effective height of mercury column. A systronics digital pH meter- 335 was used for the pH measurements. Experimental sets each having different but known amount of Cu(II) were prepared in appropriate quantity of supporting electrolyte Ammonium Buffer and pH was adjusted to 10.4 ±0.2 and titrated separately against the standard solution of the titled 6-thioguanine whose pH was also adjusted to that of the titrate (10.4± 0.2 using NaOH / HCl) at -0.04 V Versus SCE The plateau potential of Cu(II) The current offer each addition of the titrant was read and a curve was plotted between current against volume of titrant added

2.2. Synthesis Procedure of Solid Complex

Copper sulphate and 6-thioguanine were prepared separately in water and were mixed in 1:1 molar ratio the mixture was then refluxed in a round bottom flask for 2h. The complex was marked by precipitation after reducing (complex) was filtered and washed thoroughly to remove any unreacted material, the complex was dried at low temperature and store over P_4O_{10}. The results of elemental (C, H, N) and O analysis on the drug and Cu(II)- 6-thioguanine complex was furnished by CDRI Lucknow, India. The gravimetric method was used for the estimation of Copper in complex [11]. Infrared spectra were collected using a Bruker IFS66 spectrometer equipped with a Spectra-Tech Diffuse Reflectance Accessory (DRA), Sydney, Australia. The spectrometer is equipped with the following: an air-cooled DTGS detector, a KBr beamsplitter with a spectral range of 4000 to 650 cm^{-1}.

2.3. Antimicrobial Screening

The microorganisms used in this study were *Klebsiella pneumonia, Proteus. m, Streptococcus aureus*, *Asperginus niger* and *Nigrosporas S.P*. All strains were obtained from the microbiology department, Govt. Motilal Vigyan Mahavidyalaya, Bhopal (M.P.) Indian. Each Microorganism maintained on Meuller-Hinton (MH) agar medium at 4ºC

Kirby-Baller *et al.* disc diffusion was followed for the antimicrobial activity screening of the complex against various microorganisms: *Klebsiella pneumonia, Proteus. m, Streptococcus aureus*, *Asperginus niger* and *Nigrosporas S.P* [12]. The number of replicates in each case was three and the percentage of inhibition was calculated using the following Formula [13].

$$\text{Percentage inhibition} = \frac{a-b}{a} \times 100$$

Where a, represents the diameter of inhibition zone for control 6-thiguanine and b represent the diameter of inbibition zones of complex (Cu(II) 6-thioguanine)

2.4. Pharmacological Studies

In-vitro and *vivo* study of anticancer activity of prepared metal drug complex have been done using the following procedure [14-16].

2.4.1. In Vitro

B16-F10 murine melanoma tumor cell line strains was purchased from Jawaharlal Nehru Cancer Hospital and Research Centre (JNCHRC), Idgah Hills, Bhopal M.P, India as a monolayer culture in Roux battles (Coring plastics U.S.A). The cells B16-F10 (17) melanoma obtained were culture in 5ml 24 well culture plate (corning plastics, USA). The cells were seeded in 2×10^5 cell per well were grown in 1.0 ml dulbecco's modified Eagles medium (DMEM) supplemented with10% fetal bovine serum (FBS), 1% non-essential amino acid. 1mM sodium pyruvate, 100 µg/ml penicillin, 100 µg/ml streptomycin and 5% v/v heat inactivated foetal calf serum. The B16F10 cell line was growth at the cells were kept in incubator at 37°C for 8h in 5% CO_2 atmosphere and 95% humidity. The cell counter was made on Neubaurs Chamber (Fine optic, Germany).

Two dilutions viz, 1µm, 10µm of pure drug and its complex was made and then the cells were treated as follows

Column	Free Drug	Metal Complex
A	1µm(1ML)	1µm(1ML)
B	10µm(1ML)	10µm(1ML)

After addition of the respective solutions, the culture plate was incubated at 37°C for 8 hours. Finally, the cell counts and viability were conducted under microscope after trypan blue staining and compared to

the cell cultured in DMEM medium without treatment as control.

Cells Viability Counts

Cell Viability counts were made by trypan blue dye exclusion test. Two drops of trypan blue were added to each cell culture well and kept for 15 minutes. Now a drop of culture was added to hemocytometer (Neubaurs chambers, Grenoble, France) and the number of stained, non stained and total numbers of cells were counted, then the % inhibition was calculated using the following equation:

% inhibition =,No. of viable cells before treatment−No. of viable cells after treatment -No. of viable cells before without treatment . × *100*

The experiment of each concentration of the drug and the complex was repeated three times and statistical conclusions were drawn.

2.4.2. In Vivo

Experiments were performed on Male C57BL/6J black mice, 6-8 weeks old, weighing 25-30 gm purchased from laboratory animals center Jawaharlal Nehru Cancer Hospital & Research Centre (JNCHRC), Idgah Hills, Bhopal, India. Mice were kept in cages with sawdust bedding and given food and water ad libitum air conditioned animal house. The comparative efficiency of pure and complex form of 6-thioguanine drug were evaluated from the difference in response after treatment with two forms of drug.

Animal model: C57BL/6 Mice weight 25 – 30 gm

Tumore model: B16-F10 Melanoma cell Line.

Drug: 6-thioguanine and it's Copper Complex.

Cell growing in a nutrient medium (DMEM) were obtained from NCCS Pune . They were brought into a single cell suspension by trypsinization (0.2% trypsin).The cell suspension was centrifuged to obtain concentrated suspension (2×10^5 cell/ml) approximately 10^5 cells of tumor were injected on the dorsal skin of adult C57BL/6 mice and allowed to tumor to grow palpable size was reached by 6-8 days.

The time required to double the tumor volume (volume doubling time (VDT) from 100 to 200 mm^3 was taken as a criterion to assess the antitumor efficiency of pure and complex drug in B16F10 tumor bearing mice. The treatment was started after tumor size reached 100±10mm^3 Indicated dose (0.2 mg) of free drug and drug complex were injected intravenously and tumor growth was monitored. Tumor size was calculated by Formula $V=(\pi/6)\ D_1D_2D_3$ where $D_1D_2D_3$ = diameters in three perpendicular planes, were measured using a vernier caliper and V=volume of tumor [18].

3. RESULTS AND DISCUSSION

3.1. Polarographic Behaviour of 6-thioguanine with Cu(II)

In 0.1 M KCl at pH 10.4±0.2 the Cu(II) and its complex with ligand under study were found to be reversible and diffusion controlled polarographic wave which revealed by the log polt slop id versus √h (

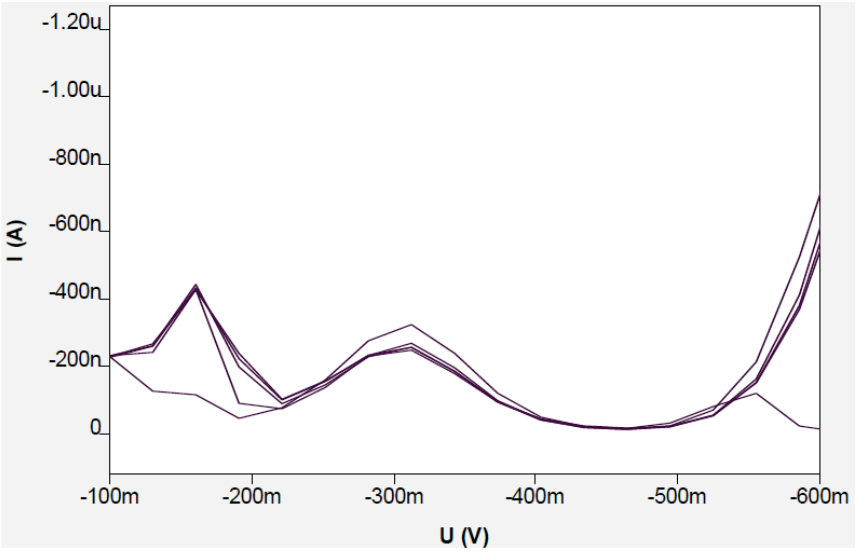

Figure 1: Polarogram of Cu(II) (2.0 mM) in 0.1M Ammoniam buffer Solution at pH 7.0±0.1 and 2.0 mM 6-thioguanine.

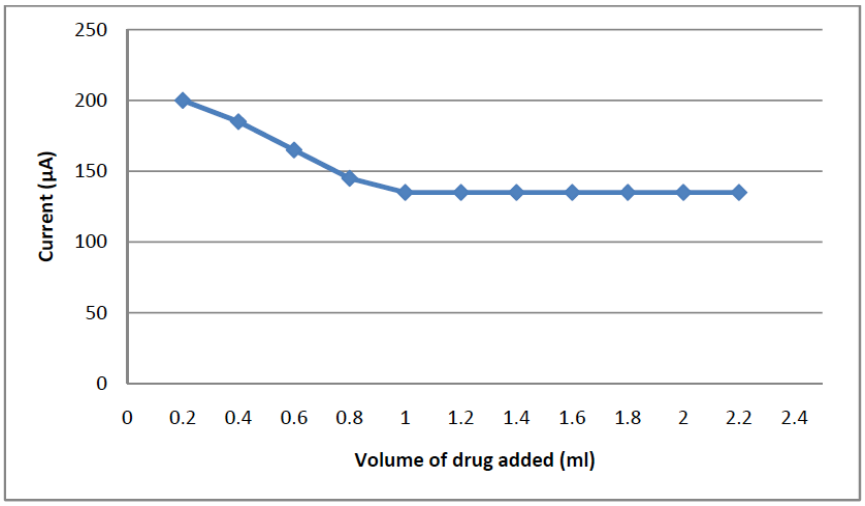

Figure 2: Amperrometric titration of (2mM/10ml) 6-thioguanine (2mM/ml) Cu (II) solution in 0.1 M Ammoniam buffer Solution.

effective height of mercury column) respectively on gradual addition of ligand the $E_{1/2}$ of metal shifted towards more electronegative value indicating the formation of complex (Figure **1**). Lingane's treatment [19] of observed polarographic data revealed 1:1 [M: L] Complex formation in solution with log β_1=6.62.

3.2. Amperometric Determination of 6-thioguanine with Cu(II)

Cu (II) with 6-thioguanine gives a well defined polarographic waves / peak in 0.1 M KCl at 10.4±0.2 pH the diffusion current was found proportional to the concentration of Cu(II). The platue potential for the polarographic wave of Cu (II) (-0.40V) Vs Hg Pool was applied for carrying out amperometeric titration. The Current goes on decreasing to minimum and then attends a constant value. The plot of id versus volume (V+vV) of titrant added, revealed L shaped curve (Figure **2**). The end point was indicated by the intersection of the two lines, which confirmed 1:1 [M: L] complex formation.

3.3. Elemental Analysis

Elemental analyses were carried out on a model 240 Perkin elemental analyzer, Massachusetts USA. Metal contents were determined gravimetrically. Percentage of 6-thioguanine drug found and Calculated in % C 35.92, H 3.01, S 19.8, N 41.89; found C 35.87, H 3.08, S 18.89, N 41.83% and its complexe with Cu are Calculated in % C 26.04, H 2.18, S 13.9, N 30.37, Cu 27.56, found C 26.14, H 2.20, S 14, N 30.29, Cu 27.4 and reaction of 6-thioguanine with metal ion in near quantitative yield are good agreement with each other elemental analysis.

3.4. Spectrometric Measurement

6-thioguanine metal complexes IR data (KBr, cm^{-1}): 3427(w), 3283(w), 3109(vs), 2929(w), 2845(w), 1665(s), 1618(s), 1539(m), 1483(w), 1440(m), 1376(m), 1259(m), 1231(m), 1143(w), 1107(w), 1031(m), 1016(m), 972(m), 871(w), 822(s), 779(w), 718(m), 621(m), 586(w), 565(w). The IR spectrum exhibits some minor perturbation in C=S vibration. The band at 1231 Cm^{-1}, attributed to C=S stretching, decrease in intensity and band at 1202 Cm^{-1}. The decrease intensity of the C=S band has been accounted for as the substitution on sulfur by metal coordination [20-21]. Furthermore, around 1600 Cm^{-1}, where the C=N and C=C appear band at 1637 Cm^{-1} and one at 1618 Cm^{-1} decrease in intensity. Based on this information, we believe the thioguanine is likely to be engaged in coordination to Cu(II) center probably assisted by weak S-Cu interaction shown in Figure **3**.

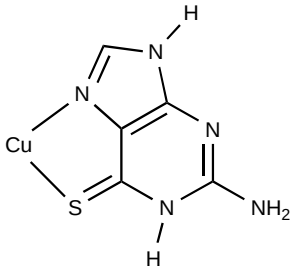

Figure 3: Structure of Cu(II)-6-thioguanine complex.

3.5. Antimicrobial Activity

Antimicrobial behavior of Cu(II) -6-thioguanine complex against various pathogenic bacteria and fungi has been reported in the (Table **1**). A perusal of data in table reveals that complex shows increased toxic

Table 1: Result of Antibacterial Activity of 6-Thioguanine and It's Cu(II)-6-thioguanine Complex

Test Organism	Zone of inhibition		% Inhibition
(A-*Bacteria)*	Control (mm)	Complex (mm)	
Streptococcus aureus	8	11	-37.5
Klebsiella pneumania	-	2	-
Proteus. M	6	10	-66.6
(B-Fungi)	7	9	-28.57
Asperginus niger			
Nigrosporan SP	6	8	-33.3

effects against all the pathogenic bacteria under study, as compared to the parent drug 6-thioguanine.

3.6. Pharmacological Studies

3.6.1. In Vitro

The result of *in-vitro* experiments of pure drugs and its complex are shown Table **2**. Perusals of the data it is compared shown that copper 6-thioguanine complex was found to be more effective than pure drug. The complex under study showed an increased inhibition against the melanoma cell line B16F10 at all the test concentrations i.e. 1, 10, µm/ML. The increased inhibition activity of complex was 26.68 ± 1.15%, 53.08 ± 1.70% as against 19.98 ± 0.43, 41.97 ± 0.98 shown by the drug, respectively. The data were statistically significant as at P< 0.05.

3.6.2. In Vivo

The results of the average of mice tumor against 6-thioguanine drug and copper complex under study are shown in Figure **4**. The results indicated that the tumor volume was 0.2cm^3 on the tumor cells injected mice without administering drug or complex after 20 days tumor size which was reduced 0.59+0.08cm^3 on tumor injected mice who were also administered the 6-thioguanine drug. The inhibition rate(IR) were 78.92% found. However in case of Cu(II) 6-thioguanine administrated mice (tumor cell injected) shows significant decrease in the tumor volume of 0.53± 0.09 cm^3 was observed and in the inhibition rate(IR) were 80.72%. Thus indicating the increasing *in vivo* tumor inhibition power of the complex over drug under study in experiment.

Table 2: *In-Vitro* Cytotoxicity of 6-thioguanine and Cu(II)-6-thioguanine Complex Against B16-F10 Melanoma Cell

Compound	Concentration µM/ml	% inhibition after 8h
6-thioguanine	1.0 10	19.98 ± 0.43 (a) (b) 41.97 ± 0.98
Cu (II)- 6-thioguanine Complex	1.0 10	26.68 ± 1.15 53.08 ± 1.70

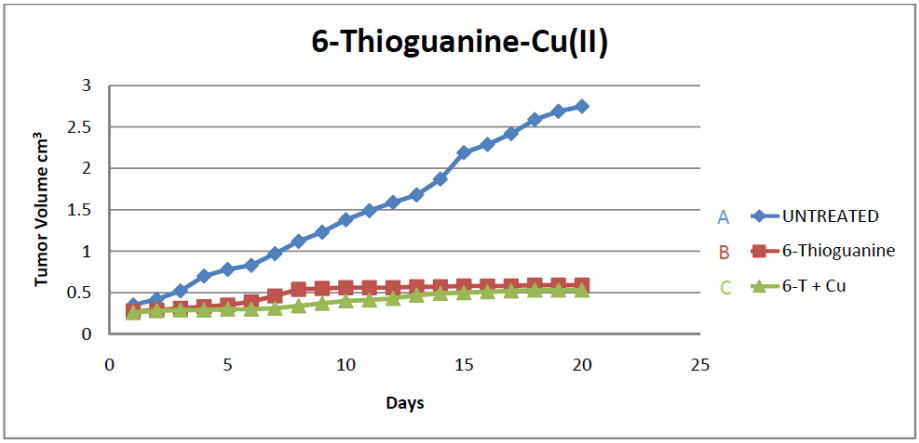

Figure 4: Effect of Cu(II)-6- thioguanine complex on tumor volume. (A, Without drug; B, With 6-thioguanine; C, With Cu(II)-6-thioguanine).

4. CONCLUSION

To investigate the structure and behaviour of complex of 6-thioguanine with life essential metal ion Cu(II) some physicochemical method i.e. IR spectral analysis,elemental analysis, amperometry and polarography have been successfully used. The obtanined of these method suggested that complexes having more stable as compared to pure drug. On the basis of observed results of pharmaceutical study Cu(II) with 6-thoguanine complex it could be concludade that drug complex with life essential metal more effective and non toxic in nature as compared to the parent drug. Thus polarographic and amperometric method may be recommended as more potent drug in lieu of the drug taken for present study have excellent potential for clinical appilication.

REFERENCES

[1] Nelson JA, Carpenter JW, Rose LM, Adamoson DY. Mechanisms of action of 6-thioguanine 6-mercaptopurine and 8 azaguanine. Cancer Res 1975; 32: 2872-78. http://dx.doi.org/10.1590/S0103-50532008000300006

[2] Ensafi AA, Hajian R. Simultaneous determination of captopril and thioguanine in pharmaceutical compounds and blood using cathodic adsorptive stripping voltammetry. J Braz Chem Soc 2008; 19: 405-12. http://dx.doi.org/10.1590/S0103-50532008000300006

[3] Anbu S, Shanmugaraju S, Ravishanker R, Karanda AA, Mukherjee PS. Naphthylhydrazone based selective and sensitive chemosensors for Cu^{2+} and their application in bioimaging. Dalton Trans 2012; 41: 13330-37. http://dx.doi.org/ 10.1039/C2DT31335A

[4] Belichi-Ferrari M, Bisceglie F, Pelosi G, *et al*. Synthesis, characterisation, X-ray structure and biological activity of three new 5-formyluracil thiosemicarbazone complexes. J Inorg Biochem 2001; 83: 169-79. http://dx.doi.org/10.1016/S0162-0134(00)00181-1

[5] Belichi-Ferrari M, Biscegliea F, Favaa GG, *et al*. Synthesis, characterization and biological activity of two new polymeric copper(II) complexes with aketoglutaric acid thiosemicarbazone. J Inorg Biochem 2002; 89: 36-40. http://dx.doi.org/10.1016/S0162-0134(01)00371-3

[6] Arguelles MR, Belichi-Ferrari M, Biscegli F, *et al*. Synthesis characterization and biological activity of Ni Cu and Zn complexes of isatin hydrazones. J Inorg Biochem 2004; 98: 313-21. http://dx.doi.org/10.1016/j.jinorgbio.2003.10.006

[7] Belichi-Ferrari M, Biscegli F, Buschin A, *et al*. Synthesis structural characterization and antiproliferative and toxic bio-activities of copper(II) and nickel(II) citronellal N^{4-} ethylmorpholine thiosemicarbazonates. J Inorg Biochem 2010; 104: 199-206. http://dx.doi.org/10.1016/j.jinorgbio.2009.11.002

[8] Lee PF, Yang CT, Fan D, Vittal JJ, Ranford JD. Synthesis, characterization and physiccochemical properties of copper(II) complexes containing salicylaldehyde semicarbazone, Polyhedron 2003; 22: 2781-86. http://dx.doi.org/10.1016/S0277-5387(03)00402-9

[9] Patole J, Dutta S, Padhey S, Sinn E. Tuning up superoxide dismutase activity of Copper complex of salicylaldehyde semicarbazone by heterocyclic bases pyridine and N- methyl imidazole. Inorg Chim Acta 2001; 318: 207-11. http://dx.doi.org/10.1016/S0020-1693(01)00416-9

[10] Reddy KH, Reddy PS, Babu PR. Synthesis, spectral studies and nuclease activity of mixed ligand copper(II) complexes of heteroaromatic semicarbazones / thiosemicarbazones an pyridine. J Inorg Biochem 1999; 77: 169-76. http://dx.doi.org/10.1016/S0162-0134(99)00188-9

[11] Bassett J, Denny RC, Jeffery GH, Mendam J. Editor Vogels Text Book of quantitative inorganic Analysis. 6th ed. ELBS Longman. Gr. Ltd. London 1996.

[12] Bauer AW, Kirby WMM, Sherris JC, Turck K. Antibiotic Susceptibility testing by standardized single disk method. Am J Clin Pathol 1966; 36: 493-96. http://dx.doi.org/10.4314/ahs.v12i2.9

[13] Smith AL. Principles of microbiology. 7th ed. The L V Mosky company, saint: Louis 1973.

[14] Layek B, Mukherjee B. Tamoxifen Citrate Encapsulated Sustained Release Liposomes: Preparation and Evaluation of Physicochemical Properties. Sci Pharm 2010; 78: 507-15. http://dx.doi.org/10.3797/scipharm.0911-11

[15] Ghosh MN. Fundamentals of experimental pharmacology. Scientific Book Agency, 2nd ed. 1984; 153.

[16] Shrivastava S, Ganesh N. Tumor inhibition and Cytotoxicity assay by aqueous extract of onion *(Allium cepa)* & Garlic *(Allium sativum)*: an *in-vitro* analysis. Int J Phytomed 2010; 2: 80-84. http://dx.doi.org:10.5138/ijpm.2010.0975.0185.02013

[17] Hejna M, Raderer M, Zielinsk CC. Inhibition of Metastases by Anticoagulants. J Nat Cancer Inst 1999; 91(1): 22-36. http://dx.doi.org:10.1093/jnci/91.1.22

[18] Rajanaresh RA, Udupa N, Uma DP. Toxicity and antitumor activity of niosomal bleomycin in tumor bearing mice. Indian J Expt Biol 1996; 34: 764-72. http://dx.doi.org/10.1515/BC.2004.081

[19] Lingane JJ. Interpretation of the Polarographic Waves of Complex Metal Ions Chem Rev 1941; 29(1): 1-35. http://dx.doi.org/10.1021/cr60092a001

[20] Casas JS, Castellano EE, Garcia-Tasande MS, *et al*. Deprotonation reactions of 2- thiouracil with [2-(pyridin-2-yl-) phenyl]mercury(II) acetate. Structural and spectroscopic effects. J Chem Soc Dalton Trans 1996; 9: 1973-78. http://dx.doi.org/10.1039/DT9960001973

[21] Melendez E, Marrero M, Rivera C, Hernandez E, Segal A. Spectroscopic characterization of titanocene complexes with Thionucleobases. Inorganica Chimica Acta 2000; 298: 178-86. http://dx.doi.org/10.1016/S0020-1693(99)00441-7

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