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Research Article

CYTOTOXIC, ANTITUMOR AND ANTIMICROBIAL STUDIES OF AN OXYGEN AND NITROGEN DONOR, NOVEL SCHIFF BASE LIGAND, ACETOACETANILIDE-(1,2-ETHYLENEDIIMINE) ETHYLACETOACETATE AND ITS TRANSITION METAL COMPLEXES

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ABSTRACT

The Schiff base, acetoacetanilide-(1, 2-ethylediimine)ethylacetoacetate (AcEE) and its six transition metal compounds were synthesized, characterised and tested for their Cytotoxic, anticancer, antitumor, antimicrobial activities. The ligand AcEE synthesized from acetoacetanilied and ethylacetoacetate with ethylenediamine. Daltons Lymphoma Ascites cell (DLA) induced solid and Ehrliche`s Ascites Carcinoma cell (EAC) induced ascites tumour models were used for antitumor studies of the compounds. The ligand, AcEE and its complexes were screened against *C. albicans*, *C. Tropicalis* and *A. Flavus* fungi and *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* bacteria to evaluate their potential antimicrobial activities. The resultant data are quite propitiating. The copper complex was found to have higher IC_{50} value, 49 µ/ml. Thus, it was found that the AcEE of Cu(II) complex was effective in raising the average life span of the tumour enduring mice by 36.6, 17.9 and 13.6 % at 5, 10 and 15 mg/kg doses respectively It is clear from the antifungal screening data that the metal complexes are more fungitoxic than the chelating agent itself. The antibacterial activity results revealed that the ligand, AcEE has more sensitive and potent for gram-positive than gram-negative bacteria.The Schiff base, AcEE and its six transition metal complexes were synthesized, characterised and tested for their Cytotoxic, anticancer, antitumor, antimicrobial activities. The complexes especially Cu(II) AcEE was found most active among all the synthesised compounds.

Key words: Acetoacetanilide-(1, 2-ethylediimine)ethylacetoacetate, Trypan blue exclusion method, Survival rate, Micro-both dilution method, cytotoxicity, antimicrobial, antifungal

INTRODUCTION

Compounds having an azomethine group (-CH=N-), known as Schiff bases[1-3] are readily formed by the condensation of a primary amine with a carbonyl compounds (aldehyde or ketone compounds)[4-7]. Schiff`s base could be applied in different areas such as separation processes, metallic deactivation, bioinorganic chemistry, catalysis, electro chemistry and environmental chemistry[8,9]. They have immense applications in the area of farming (as pesticides) and in pharmaceutics (as antimicrobial agents)[10]. They attract many researchers because of their wide applications in cytotoxic, anticancer and pharmaceutical fields[11,12]. e.g. as anticancer agent[13], as bactericides[14], antiviral agents[15] and fungicides[16,17]. The binding capacity of Schiff bases to metals has been widely studied. But, their relationship with each other has not been studied much. In Schiff base compounds, the imines nitrogen can act as an inter- or intra-molecular hydrogen-bond acceptor. The hydroxyl oxygen in salicylaldehyde derivatives can act as intermolecular hydrogen-bond acceptor. Hydrogen bonding interactions are significant in their pharmaceutical industry applications. The nature and strength of the interactions between the molecules can influence the uptake of the medicine in the body. The actual role of metal complexes used as drugs were known only after the invention of *cis*-platin, i.e., *cis*-[dichlorodiammine] platinum(II)[18]. Carbo-platin and *cis*-platin still have important roles in cancer chemotherapy[19,20]. Since the invention of the anti-tubercular action of Schiff base metal complexes, a gigantic study has been made on the field of pharmacology of these types of compounds[21]. Literature review reported that the metal complexes especially transition metal metals compounds of Schiff bases have better antitumour properties, when compared to free ligand[22]. They act in mammalian cells by encumber the enzyme, ribonucleotide reductase, is an essential in the preparation of DNA precursors[23]. Wang M and Wang L F *et al*[24] reported that the anticancer studies of metal compounds of Co(II), Ni(II), Cu(II) and Zn(II) thiosemicarbazone derived from 3-acetylumbelliferon. Among these complexes, Co(II) and Cu(II) exhibit better inhibitory effect compared to others. The present work is an extension of our previous studies and is devoted to the preparation and identification of complexes of Cr(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) with a Schiff base ligand synthesized from ethylacetoacetate and acetoacetanilide with ethylenediamine. The characterization was done based on elemental analysis, magnetic measurements, molar conductance, IR, ^{1}H NMR and electronic spectral data. The ligand, acetoacetanilide-(1, 2-ethylediimine) ethylacetoacetate (L2H) and its copper complex were tested for their cytotoxic and antitumour activities. The ligand and its metal complexes were screened against *C. albicans, C. tropicalis*, *A. flavus*, and *A. niger* fungi and *Pseudomonas aeruginosa*, (PTCC 1074) *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1330), and *Bacillus cereus* (PTCC 1015) bacteria to evaluate their efficacious antifungal and antibacterial activities respectively.

MATERIALS AND METHODS

The metal salts used in this study were BDH AnalaR quality. For the preparation of the AcEE, acetoacetanilide, ethylenediamine and ethylacetoacetate were used. Mainly chlorides, sulphates, nitrates and acetate of Cr(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) were selected for the preparation of metal compounds. The commercially available solvents, ethanol, methanol, chloroform, DMF, DMSO, petroleum ether, diethyl ether etc. were taken for the preparation, extraction and recrystalization of the ligand and metal complexes. All the solvents except E. Merk reagent grade were purified by standard method.

Preparation of the Ligand, Acetoacetanilide-(1,2-ethylediimine)ethylacetoacetate: (AcEE))

A methanolic solution of ethylacetoacetate (0.025 mol in 25 ml) and acetoacetanilide (0.025 mol in 25 ml) were added to ethylenediamine (0.025 mol) in minimum amount of methanol in a 250 ml round bottom flask with stirring. The mixture was kept under reflux for two hours, and then the resultant yellow coloured mixture was cooled in an ice bath. The off-white product formed was filtered, washed a number of times with ethanol and finally with petroleum benzene and allowed to dried over anhydrous $CaCl_2$. Yellowish crystalline solid; yield 80 %; m. p: 145^0C; Solubility: DMSO, DMF; UV-Vis λ_{max}: 286 nm, 436 nm; IR: ν = 1612 cm^{-1} (C=O), ν = 1580 cm^{-1} $(C=N)_{azomethine}$, ν = 1515 cm^{-1} $(C=N^{I})_{azomethine}$, ν = 3300 cm^{-1} (-OH), ν = 2049 cm^{-1} (-NH), ν = 3110 cm^{-1} (Ar). (figure.1)

Preparation of metal complexes

The 0.005 mol of metal salts (Cr (III), Fe (III), Co (II), Ni (II), Cu (II) and Zn (II)) in minimum amount of ethanol was added to DMSO solution of the ligand (0.005 mol in 20 ml) in dimethyl sulphoxide (DMSO) in 1:1 molar ratio and it was kept under reflux for about 4 h. It was then cooled and allowed to evaporate. After filtering, the solid complex obtained was washed several times with petroleum benzene and finally with ethanol and dried over anhydrous $CaCl_2$. (Yield: 70–80%, m.p = 250-350^0C). (Table.1, figure.2)

Characterization of the Ligand, Acetoacetanilide-(1,2-ethylediimine)ethylacetoacetate (AcEE) and its metal complexes

The ligand (Figure.3a) and the complexes (Figure.3b) were characterized based on their elemental analysis, magnetic moment data, and IR, UV/Vis and ^{1}H NMR spectral techniques.

Assessment of anticancer potential

The Cancer Institute (WIA) at Adayar, Chennai provided the essential EAC cell lines and Dalton's Lymphoma Ascites (DLA) and disseminated as transplantable tumours in the peritoneal cavity of BALB/C mice. The NCCS, is a national level biotechnology, tissue engineering and tissue banking research centre, Pune, India was supplied L929 (mouse lung fibro blast) cell line for our investigation.

The Swiss albino laboratory mice (20-26 g) were acquired from the Mannuthy small Animal Breeding Station, Thrissur, Kerala. They were stored in Amala Cancer Research Centre animal house, Trissur Kerala and provided standard condition of humidity and temperature with standard food (mouse chow) and water *ad libitum*. Institutional Animal Ethics Committee (IAEC) were given the prior permission for the all animal experiments in the present investigation and carried out strictly according to the strategy of CPCSEA established by the Animal Welfare Division, Government of India.

Mouse lung fibroblasts (L929 cells) were allowed to culture in DMEM medium supplemented with FBS (10% v/v), streptomycin (100 μg/ml) and penicillin (100 U/ml) and stored at 37^0C in an incubator with 5% carbon dioxide. DLA and EAC cells were perpetuated in mouse (intraperitoneal cavity) were treated for the experiment.

The synthesis of the drug

To 1 ml of the dimethylsulphoxide (DMSO) added 50 milligrams of the sample (Ligand and metal complexes) and dissolve. Using the solution *in vitro* studies was conducted. For conducting *in vivo* studies, 50 mg of the sample was first dissolved in 1 ml DMSO and it was further diluted with distilled water to the desired concentration.

Trypan blue exclusion method

Using DLA cells, the compounds, to be tested, were investigated for short-term *in vitro* cytotoxicity studies. The cancer affected tumour cells for conducting the study aspirated from the peritoneal cavity of tumour bolstering mice where washed three times using PBS (phosphate buffered saline). Trypan blue exclusion method was used for the determination of Cell viability. The suspension of viable cell (1×106 cells in 0.1 ml) was added to test tubes enclosing various concentrations of the test samples and the volume was made up to 1 ml with PBS. The test tubes labelled as control consist of the cell suspension only. These samples and control to be analysed were allowed to incubate at 37^0C for 3 hours. 0.1 ml of 1% Trypan blue was added to the cell suspension and placed for 2 to 4 minutes and loaded on a haemocytometer. It was observed that, the dead cells appeared in blue colour, due to the absorption blue stain of Trypan blue, while live cells did not absorb the dye. The number of each stained and unstained cells were estimated separately.

Toxicity analysis Schiff base and its metal complexes

30 laboratory Swiss albino mice were categorized into 5 groups (6 mice/group). Group 1, 2, 3 and 4 were treated with 5mg/kg, 10 mg/kg, 15 mg/kg and 20 mg/kg respectively. The group 5 was treated as control. The drug was given to the mice once in a day by intraperitoneal injection and continued for 6 weeks. The mortality rate of the animals was noted.

Effect of acetoacetanilide-(1, 2-ethylediimine) ethylacetoacetate copper complex

Survival rate of ascites tumour enduring Swiss albino mice

Mice (female, 35 – 56 days old) weighted 26–30 g were spitted into 5 groups having 6 animals per group. Viable EAC cells 106 in 0.1 ml of PBS were administrated by injecting on the peritoneal cavity of the animal. Group 1 and 5 were treated as control and standard (cyclophosphamide) respectively and group 2, 3 and 4 treated with 5mg/kg, 10 mg/kg and 15 mg/kg respectively.

Drug and standard drug cyclophosphamide were administrated by intraperitoneal injection to the animal from the first day of tumour induction. The mortal rate of mice due to tumour encumbrance was recorded and percentage of increase in life span (ILS) was determined as, % ILS= (T-C/C) ×100, where T

and C are mean survival of treated and control mice, respectively.

On solid tumour development

Swiss albino mice (35 – 56 days old) weighing 23–28 g were splitted into five groups, each group composed of 6 animals for the above studies. DLA cells (0.1 ml of 106 cells per mouse) were administrated by injection in to the right hind leg of mice to induce tumour. Group 1 was treated as control animals. Copper complex of Schiff base AcEE was given to the 2, 3 and 4th groups for treatment. Group 5 was taken as standard animals and treated with standard drug cyclophosphamide. The tumour growth on the mice of each group was arbitrated by estimating the diameter of tumour volume in two perpendicular planes using a digital vernier calliper, starting from 7th day of tumour growth up to 31th day. The volume of tumour development was calculated using the equation, $V=4/3\pi r_1^2 r_2$, where r_1 is the minor diameter and r_2 is the major diameter[25].

Antifungal activity

For the isolation of fungi, dilution plate method[26] was used. Selected and isolated fungi were maintained on potato dextrose agar plates at 4°C for further experimental work. The antifungal studies of the ligand (AcEE), its complexes, fungicides (bavistin and emcarb) and the control DMSO (dimethyl sulfoxide) were screened using the plate poison technique. Seven day-old cultures of *C. Albicans, C. Tropicalis* and *A. Flavus* were used as test organisms. A stock solution of 500 g/ml was made by dissolving 50 mg of each compound in DMSO (100 ml). The sterilized medium with the stock solution was added into 90 mm sterile petri plates and kept for solidification. They were inoculated with a 5-mm actively growing mycelia disc and incubated at 27°C. After 72 h of inoculation, the percentage reduction in the radial growth diameter over the control was calculated. The growth was compared with dimethylsulfoxide as the control.

Antibacterial activity

In this study, we used four bacteria (two gram-positive bacteria and two gram-negative bacteria). The standard strains of the following microorganisms were used as test organisms *Pseudomonas aeruginosa,* (PTCC 1074) *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1330), and *Bacillus cereus* (PTCC 1015). Bacterial isolates were developed in each medium for 24 h at 37°C. The inoculums density of each bacterial isolate was standardized with 0.5 McFarland turbidity standards. The suspension had a final inocolumn of 5 × 108 cfu/mL (colony-forming unit). Two methods, disc diffusion and micro-broth dilution methods were used to test antibacterial activity. (Table.8)

Disc diffusion method

We prepared and sterilized the Müller Hinton agar medium (38 g Müller Hinton agar and 3 g agar agar in 1000 mL of distilled water). A small amount of each bacteria was placed on the side of the plate. Using a sterile loop spread the bacteria in one direction from the starting site of inoculation. The plates were allowed to incubate at 37°C for 24 hours for bacterial growth. A bit of each bacteria was added in a sterile distilled water tube similar to 0.5 McFarland turbidity standard (the suspension had a final inoculum of 5 × 108 cfu/mL). The plates (with respect to the number of samples) were inoculated with bacteria by two sterile cotton swabs. The substance (0.02 g) was dissolved in 1 mL of DMSO. The sterile blank discs (Whitman no. 1 filter paper, 5 mm diameter) were dipped in 0.1 mL of each sample. The discs were placed on plates at specified intervals by sterile forceps. After an incubation period at 37°C for 24 hours, the diameter of each zone of inhibition was measured with a ruler (mm). The standards used for antibacterial measurement were Imipeneme (10 µg per disc), Ampicillin (10 µg per disc) and Chloramphenicol (30 µg per disc). To clarify any participating role of DMSO in the biological screening showed no activity against any bacterial strains. The test results are presented in Table 6. These results were confirmed by repeating the test three times using the same procedure conditions.

Micro-broth dilution method for MIC

The MIC (Minimum Inhibitory Concentration) is the lowest concentration of the test compound. 13 sterile tubes containing 1 mL of the solution of Müeller nutrient broth medium were prepared. Each compound (0.02 g) was dissolved in 1 mL of DMSO. Then the first tube was filled with 1 mL of the test sample. 1 mL of the solution from the first tube was pipetted out and added to the second tube. Then, 1 mL of the solution from the second tube was pipetted out and added to the third tube. This process was repeated for all the 12 tubes. As a result, the concentration in each tube will be half of the previous tube. The extra solution (1 mL) from the 12th tube was discarded. Thus, the 13th tube acted as control bacteria. After 24 h of incubation at 37°C, a bit of one bacteria was dissolved in a sterile distilled water tube similar to 0.5 McFarland turbidity standard. A specified amount of bacterial suspension was added in all tubes except to the 12th tube (as a control sample) until the concentration in all the tubes was 5 × 105 cfu/mL (Colony forming unit per millilitre) and incubate at 37°C. After 24 hours, MIC (Minimum Inhibitory Concentration) values of the substances were determined by the control tubes. MIC values of the ligand, AcEE and its metal compounds are given in the Table 7.

RESULTS

Short-term *in vitro* cytotoxic analysis of the ligand, AcEE and its metal complexes

The ligand, AcEE and its Cr(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) compounds exhibited striking cytotoxic activity against DLA cell line (Table 2). The Cu(II) AcEE complex exhibited highest activity with an IC_{50} (The concentration required for 50% death) value of 49 µg/ml (Table.2)

Toxicity studies

The findings of toxicity studies of Cu(II) AcEE complex on 24 Swiss albino experimental mice of four groups, at four different concentrations (20, 15, 10 and 5 mg/kg) exhibited that 20 mg/kg was slightly noxious to the mice. Therefore, this concentration was abstained and only 15, 10 and 5 mg/kg were elected for *in vivo* studies.

Action of Cu(II) complex of AcEE on ascites tumour growth

The tumour enduring Swiss albino mice of the control group survived for a period of 15.4 days. That group, treated by standard drug cyclophosphamide survived for 25.8 days. Those group treated by Cu(II) AcEE complex at 15, 10 and 5 mg/kg concentrations raised the survival rate of mice by 17.5, 18.1 and 21 days, respectively (Table 3). Thus, it was found that the AcEE of Cu(II) complex was effective in raising the average life

span of the tumour enduring mice by 36.6, 17.9 and 13.6 % at 5, 10 and 15 mg/kg doses respectively (Table 4).

Effect of Cu(II) AcEE complex on reduction solid tumour volume

Tumour volume of animals in the control group, enlarged by 2.973 cm^3 on 31th day, while treated with the Cu(II) AcEE complex, there was a significant decrease of tumour volume. At 5 mg/kg, the volume was 1.429 cm^3, while at higher concentrations (15 and 10 mg/kg) the tumour volumes were found to be 1.844 and 1.742 cm^3, respectively. Treatment with standard drug, cyclophosphamide, reduction in tumour volume was 0.698 cm^3 (Table 5, figure.5)

Antifungal studies

The free Schiff base ligand, AcEE and its metal compounds were screened against *C. albicans, C. Tropicalis* and *A. flavus* fungi and the results were quite promising. It was apprehensible from the antifungal screening study (Table.6) that the metal compounds of AcEE were better fungitoxic than the ligand itself. It has been also observed that higher activity against A. Flavus and medium activity against *C. albicans* and *C. Tropicalis*. The complexes also exhibit species-dependent antifungal activity (Table 6).

Antibacterial study

The AcEE and its metal compounds were screened against *S. aureus B. Cereus, P. Aeruginosa* and *E.coli* bacteria to evaluate their potential antimicrobial activity. The antibacterial activity results (Table. 7) revealed that the free ligand, AcEE has more potent for gram-positive than gram-negative bacteria. The biological activity of the transition metal AcEE complexes follows the order Cu(II) > Ni(II) > Fe(III) > Co(II) > Cr(III). Thus the metal compounds were found to have higher biological activity than the parent Schiff base (Table 7 and 8) towards both gram-positive and gram-negative bacteria. This showed that the incorporation of metal ions in chelation can increase the antibacterial action of the parent organic ligand compounds.

Table 1: Analytical and physical properties of ligand (AcEE) and metal complexes

Si. No	Compound	Melting point (^{0}C)	Elemental %, Found (Calculated)				Colour
			Metal	C	H	N	
1.	L2H	145	---	53.99 (54.55)	5.06 (5.85)	9.96 (10.60)	Off-white
2.	[CrL(H_2O)Cl]	<300	12.00 (12.46)	51.01 (51.79)	5.93 (6.52)	9.97 (10.07)	Greenish - brown
3.	[Fe(L2H)Cl_3]	298	10.96 (11.31)	48.00 (48.80)	6.00 (5.10)	7.99 (8.51)	Dark red
4.	[CoL]	275	15.00 (15.88)	56.01 (56.67)	6.01 (5.97)	11.00 (10.82)	Light red
5.	[NiL(H_2O)$_2$]	265	14.01 (13.84)	51.00 (50.97)	6.05 (6.42)	10.01 (9.92)	Green
6.	[CuL(H_2O)$_2$]	290	13.99 (14.62)	51.05 (50.19)	5.95 (6.27)	10.00 (9.75)	Off-blue
7.	[ZnL]	<300	15.00 (16.16)	53.99 (54.76)	5.02 (5.87)	9.70 (10.64)	White

Table 2: Percentage of cytotoxicity of ligand and complexes

Concentration (µg/ml)	Percentage of Cytotoxicity						
	Complexes						Ligand (AcEE)
	Cr	Fe	Co	Ni	Cu	Zn	
200	55	72	55	78	85	26	34
100	42	53	42	60	72	18	25
50	30	40	30	57	50	12	14
20	22	35	22	36	30	6	10
10	10	30	10	28	20	4	5

Table 3: Effect of copper complex of AcEE of survival rate of ascities tumour enduring mice

Treatment (mg/kg)	Survival rate (Days)
Control	15.4
15	17.5
10	18.1
05	21
Standard*	25.8

*cyclophosphamide (10)

Table 4: Effect of copper complex of AcEE on the life span rate of ascities tumour enduring mice

Treatment (mg/kg)	Increase in average Life span (%)
Control	--
15	13.6
10	17.9
05	36.6
Standard*	67.5

* cyclophosphamide (10)

Table 5: Effect of copper complex of AcEE on the reduction of tumour volume

Dosage (mg/kg)	Observation (No: of days)								
	Initial	**10**	**13**	**16**	**19**	**22**	**25**	**28**	**31**
Mean volume	0.094	0.701	0.990	1.421	1.982	2.341	2.681	2.994	3.001
15	0.080	0.510	0.591	0.682	0.834	0.884	0.992	1.120	1.844
10	0.079	0.423	0.423	0.794	0.844	0.923	1.024	1.342	1.742
05	0.078	0.484	0.484	0.642	0.714	0.794	0.948	1.012	1.429
Standard	0.080	0.214	0.214	0.248	0.304	0.400	0.490	0.514	0.698

Table 6: Antifungal studies of ligand and metal complexes

Compounds	Fungi, % Inhibition (growth diameter in mm)		
	C. albicans	***C. Tropicalis***	***A. Flavus***
Emcarb*	(00)100	(00)100	----
Bavistin*	(00)100	(00)100	(00)100
DMSO (control)	(21)29	(28)33)	(32)40
Ligand	(12)15	(12)14	(15)19
FeL	(23)24	(18)26	(15)25
CoL	(14)24	(23)26	(17)21
NiL	(19)28	(26)27	(17)23
CuL	(20)32	(21)29	(24)39

* Conventional fungicides.

Table 7: Inhibition zones (mm) of complexes and ligand against bacterial strains

Compounds	Bacteria (D* mm)			
	Staphylococcus aureus	***Bacillus cereus***	***Escherichia coli***	***Pseudomonas aeruginosa***
L2H	8	7	6	6
FeL	14	12	10	11
CoL	8	11	11	8
NiL	--	8	10	11
CuL	15	16	14	10
Ampicilin	14	--	12	--
Choloramphenicol	13	--	14	8
Imipeneme	20	21	24	--
DMSO	--	--	--	--

*(*D*) Diameter inhibition zone (in mm)

Table 8: Minimum inhibition concentration, mg ml^{-1}

Compounds	Bacteria (D* mm)			
	Staphylococcus aureus	***Bacillus cereus***	***Escherichia coli***	***Pseudomonas aeruginosa***
L2H	2.50	5.00	2.50	5.00
FeL	0.15	2.15	1.50	2.50
CoL	0.50	0.31	1.25	2.50
NiL	0.61	1.25	0.62	1.25
CuL	0.65	2.50	0.62	1.25

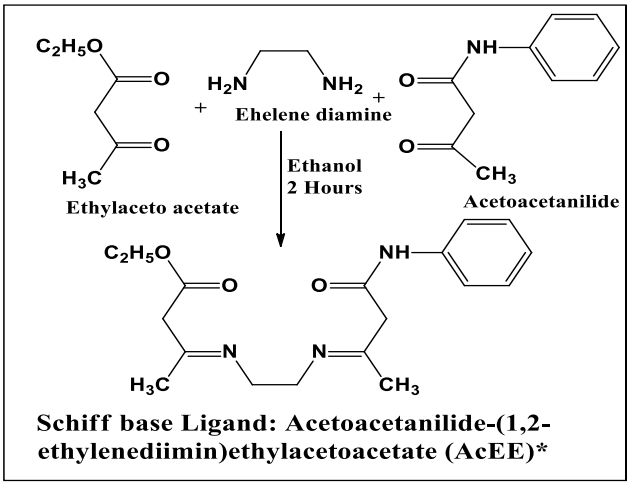


Figure. 1: Synthesis of Ligand

*IUPAC name of the AcEE Schiff base ligand: (E)-ethyl 3-((2-((E)-(4-oxo-4-(phenylamino)butan-2-ylidene)amino)ethyl)imino)butanoate

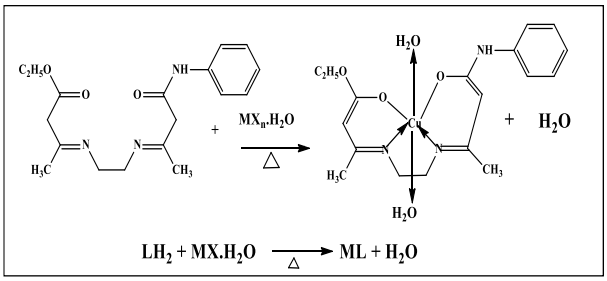
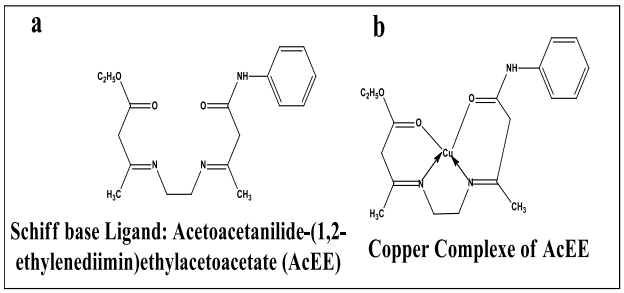


Figure.2: Synthesis of metal complexes



Figures. 3: Structures of (a). AcEE and (b). Cu(II) complex of AcEE

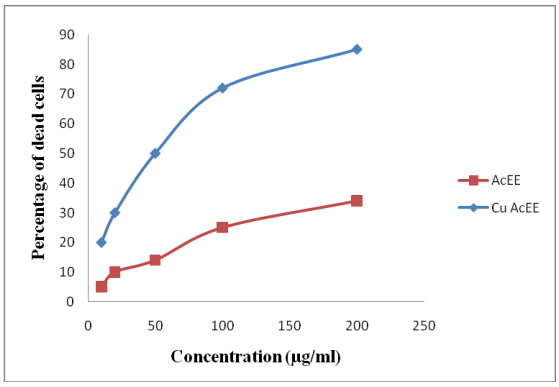


Figure. 4: Cytotoxic action of AcEE ligand and its Copper complex

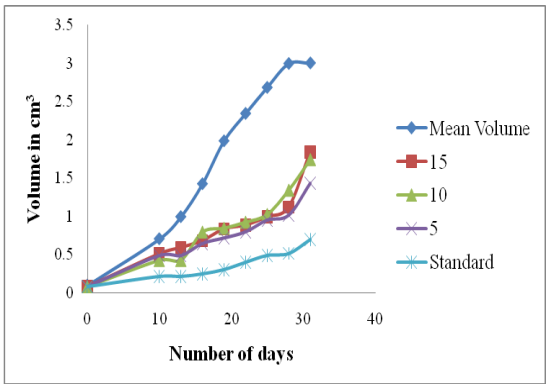


Figure. 5: Effect of copper complex of AcEE on solid tumour induced by Dalton`s lymphoma ascites cells

DISCUSSION

The N-N-S and O-N-O donor systems are characteristic features in all metal complexes, has carcinostatic potency. We have carried the anticancer studies of acetoacetanilide-(1,2-ethylediimine)ethylacetoacetate (AcEE) and its metal compounds and we got interesting and promising results. *In vitro* cytotoxicity studies on AcEE and its different metal compounds exhibited cytotoxicity against DLA cell lines. The Cu(II) AcEE complex exhibited highest cytotoxicity with an interesting IC_{50} value around 49 µg/ml.

From the present study, we concluded that the Cu(II) AcEE compound is efficient for DLA-induced solid tumour and EAC-induced ascites tumour. Among the three concentrations (5 mg/kg, 10 mg/kg and 15 mg/kg body weights), 5 mg/kg body weights was more efficient than the other two, in both cases. *In vitro* cytotoxic and antitumour activities of the Cu(II) AcEE compound, it can be suggest its potential use as an anticancer agent.

Bactericidal action of the ligand, AcEE and their transition metal compounds were screened against different bacteria and the results are shown in Table 7. It has been suggested that the ligands with the O- and N-donor systems might have inhibited enzyme production. The complexation makes their diffusion through the lipid layer of spore membranes to the site of action easy and finally killing them. The deviation in the efficacious of diverse biocidal agents against different organisms depends on the cell impermeability. The inhibition zone diameter (mm/mg sample) data of the investigated compounds are concised in Tables 7, 8. A comparative study of the diameter of inhibition zone (mm/mg sample) values of the ligand and the complexes demonstrate that the metal complexes show higher antimicrobial activity than the free ligand, AcEE. Such higher activity of the transition metal complexes can be elucidate on the basis of Tweedy's chelation theory[27,28]. Since there is a partial sharing of the positive charge of the metal ion with donor group in complex, chelation decreases its polarity. The delocalization of the π-electrons on the whole of the chelate ring supports this. The hydrocarbon acts as a lipophilic group[29] to drive the compound through the semi permeable membrane of the cell. The results revealed that the ligand has no promising activity against the bacteria.

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