ANTINEOPLASTIC ACTIVITY OF NICKEL (II)-CYSTINE COMPLEX AGAINST EHRLICH ASCITES CARCINOMA IN SWISS ALBINO MICE

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SUMMARY: Ni(II)-cystine complex was synthesized by treating saturated aqueous solutions of Ni (II) acetate and L(-) cystine (in 1: 1 molar ratio). The complex was used to study its antineoplastic activity against EAC cells in Swiss Albino mice. It was found that the Ni (II) complex restored the depleted haematological and biochemical parameters of the EAC bearing mice towards normal values and enhanced the longevity of such mice significantly. The test compound on the other hand showed negligible host toxicity. The results obtained were compared with those obtained with a standard anticancer drug bleomycin.

Key Words: Antineoplastic activity, Ehrlich Ascites Carcinoma, Ni(II) cystine complex.

INTRODUCTION

Chemicals both synthetic as well as from natural origin are now used in medicine. They are capable of affecting selective tissues and organs and also performing certain specific functions within the organism. These chemicals are being used in such a dose and condition that they do not show any evident detrimental effect to the living system. In this connection a large number of compounds containing metals (1,2) are now used as antimicrobial and anticancer agents. The anticancer properties of platinum complexes (3-6) are well known and some of which are already been used as anticancer drugs (4). Copper complexes of ATP (7,8) and also of benzohydroxamic acid 9 are found to be effective agents against Ehrlich Ascites Carcinoma (EAC) cells in mice.

Treshchalina *et. al.* (10) studied antitumour properties of mixed coordination compounds of copper(II) and α-amino acids. These informations have inspired us to search for new coordination compounds having pronounced antineoplastic activities. With this end in view we have synthesized nickel(II)-cystine complex and studied its antineoplastic activity against EAC cells in Swiss Albino mice.

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MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and the reagents used through out the investigation were of reagent grade.

Synthesis and characterization of nickel (II) cystine complex

The Ni(II) cystine complex was synthesized by the method as described below. Saturated solutions of both nickel acetate and L(–) cystine in alcohol were mixed together in 1:1 molar ratio. The mixture solution was refluxed for a period of about 6 hours. Finally the solution was distilled to reduce about one-third of the original volume. The solution was then allowed to stand for 24 hours at room temperature when a

Table 1: Effect of test compounds on EAC cell growth inhibition (in vivo)

Name of experiment	Drugs	Dose (mg/kg)	No. of EAC cells/mouse on day 5 after tumor cell inoculation	% of cell growth inhibition
Control (EAC bearing mice)	-	-	$(9.21 \pm 1.72) \times 10^7$	-
Bleomycin	Antitumor	0.3	$(0.60 \pm 0.098) \times 10^7$	93.80%
Ni(II) complex	Synthetic	10.0	$(0.74 \pm 0.087) \times 10^7$	76.18%
		5.0	$(0.86 \pm 0.037) \times 10^7$	72.10%
		2.0	$(1.12 \pm 0.062) \times 10^7$	63.64%

green crystalline precipitate appeared. The crystals were separated by filtration, washed several times with distilled water and finally with alcohol. They were dried over silica gel and stored in a desiccator. The compound was found to be partially soluble in water and alcohol. It showed decomposition above 240^{o}C and so its melting point could not be measured.

Animals

Adult Swiss Albino male mice (20-25g) were used through out the studies. They were obtained from International Center for Diarrheal Diseases Research, Bangladesh (ICDDRB). Animals were fed with standard mouse-pellet (collected from ICDDRB) and water was given in adequate.

Tumour cells

Ehrlich Ascites Carcinoma (EAC) cells were obtained by the courtesy of Indian Institute for Chemical Biology, (IICB), Kolkata, India and were maintained by weekly intraperitoneal (i.p.) inoculation of 10^5 cells/mouse in the laboratory.

Determination of median lethal dose (LD_{50})

The LD_{50} value was determined following conventional methods (11). For the purpose the test compound was dissolved in distilled water and injected intraperitoneally to six groups of mice (each containing six in number) in different doses (10, 20, 30, 40, 50 and 60 mg/Kg). LD_{50} was evaluated by recording mortality after 24 hours.

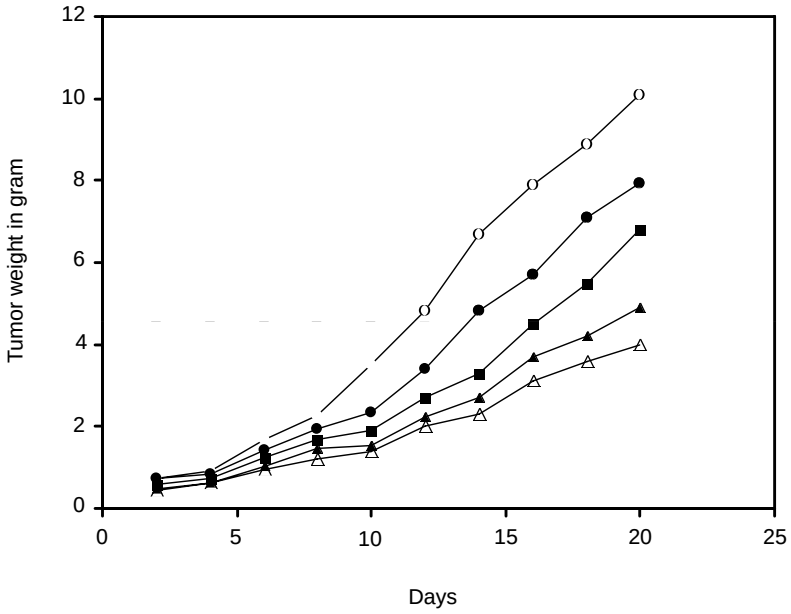
Cell growth inhibition

In vivo tumour cell growth inhibition was carried out by the method as described (12) by Sur et al. For this study 5 groups of mice (6 in each group) were used. For therapeutic evaluation 14 $\times 10^5$ cells/mouse were inoculated in to each group of mice on the first day. Treatment was started after 24 hours of tumour inoculation and continued for 5 days. Group 1 to 3 received the test compound at the doses 2 mg/Kg, 5mg/Kg and 10 mg/Kg respectively per day per mouse. In each case the volume of the test solution injected i.p. were 0.1ml/day per mouse. Group 4 received *bleomycin* (0.3 mg/kg

Table 2: Effect of Ni(II) complex on survival time of EAC cells bearing mice

Name of experiment	Drugs	Dose (mg/Kg)	Mean survival time (Days) mean ±SEM	% Increase of life span
Control (EAC Bearing mice)	-	-	9.21 ± 2.7	-
EAC+ Bleomycin	Antitumor	0.3	35.12 ± 0.95	82.82
EAC+ Ni(II) complex	Synthetic	2.0	20.82 ± 3.4	8.38
		5.0	22.9 ± 3.92	19.20
		10.0	27.8 ± 3.12	44.72

Figure 1: Effect of the test compound on the rate of tumor growth inhibition in mice. (A) Untreated EAC bearing mice, (B) 2 mg/kg, (C) 5 mg/kg, (D) 10 mg/kg and (E) 0.3 mg/kg (with bleomycin).



i.p.) and finally the Group 5 was treated with the vehicle (normal saline) and was considered as untreated control. The mice were sacrificed on the 6th day after transplantation and tumor cells were collected by repeated intraperitoneal wash with 0.9% saline. Viable tumor cells per mouse of the treated group were compared with those of control.

Average tumor weight and survival time

These parameters were measured under similar experimental conditions as stated in the previous experiment. Tumor growth was monitored daily by measuring weight change. The host survival time was recorded and expressed as mean survival time in days and percent increase of life span was calculated (13) as follows:

Mean survival time,

$$MST = \frac{\Sigma \text{ Survival time (days) of each mouse in a group}}{\text{Total number of mice}}$$

Percent increase of life span,

$$ILS\ \% = \frac{(MST \text{ of treated group})}{(MST \text{ of control group})} \times 100$$

Haematological studies

The effect of the test compound on hematological parameters in normal and EAC bearing mice was studied by the method as described in the literature (14). Four groups (with n = 4) were taken for both normal and EAC cell bearing mice, each treated with the test compound at the doses of 0, 2, 5 and 10 mg/Kg on the 12th day of tumor transplantation. Treatment was continued for 10 consecutive days. Blood was collected for individual mouse for the measurement. Total counts of white blood cell (WBC), red blood cells (RBC) and hemoglobin (Hb) content were determined by standard methods (15) using cell diluting fluids and a hemocytometer. The differential count was carried out with wright strain.

Alkaline phosphatase (ALP) activity

The ALP activity in the serum of normal and tumor bearing mice as well as of tumor bearing mice treated with the test compound was assayed by the procedure as described (16) by Michell *et. al.* on the 12th day of tumor inoculation.

Brine shrimp lethality bioassay (17,18)

Six vials were taken for this study. Exactly 5 ml of sea water (3.8% NaCl solution) was given to each of the vials. Then with the help of a micropipette specific volumes of the sample solution (4 μg/L) were transferred to each of the vials to get final sample concentrations of 0, 2, 4, 8, 16, and 20 μg/L. With the help of a Pasteur pipette, 10 living shrimps were taken to each of the vials. After 24 hours, the number of survived nauplii in each of the vials were counted.

Table 3: Effect of Ni(II) complex on haematological parameters.

Name of experiment	RBC cells/ml	WBC cells/ml	% of Hb	Lymphocytes %	Neautrophill %	Monocytes %
Normal	$(8.01 \pm 0.67) \times 10^9$	$(5.00 \pm 0.39) \times 10^6$	13.15 ± 0.12	73 ± 0.12	19 ± 1.60	9 ± 0.31
Control (EAC)	$(2.87 \pm 0.20) \times 10^9$	$(27.40 \pm 0.11) \times 10^6$	8.71 ± 0.31	39 ± 0.90	40 ± 0.13	10 ± 0.13
EAC + 2 mg/Kg	$(2.50 \pm 0.95) \times 10^9$	$(21.30 \pm 0.47) \times 10^6$	8.93 ± 0.70	51 ± 1.10	33 ± 1.20	9 ± 0.35
EAC + 5 mg/Kg	$(3.80 \pm 0.68) \times 10^9$	$(19.30 \pm 0.98) \times 10^6$	10.10 ± 0.31	54 ± 2.10	28 ± 1.75	10 ± 0.41
EAC + 10 zmg/Kg	* $(6.10 \pm 0.37) \times 10^9$	* $(14.11 \pm 0.81) \times 10^6$	* 11.00 ± 0.85	* 57 ± 0.11	* 25 ± 1.80	* 8 ± 0.38
N + 2 mg/Kg	$(6.80 \pm 0.87) \times 10^9$	$(11.01 \pm 0.10) \times 10^6$	10.97 ± 0.39	79 ± 1.10	19 ± 0.40	6 ± 0.10
N + 5 mg/Kg	$(5.40 \pm 0.92) \times 10^9$	$(6.90 \pm 0.71) \times 10^6$	10.13 ± 0.90	63 ± 0.01	16 ± 0.91	8 ± 0.61
N + 10 mg/Kg	$(5.00 \pm 0.31) \times 10^9$	$(7.82 \pm 0.80) \times 10^6$	11.05 ± 0.10	74 ± 0.30	23 ± 0.10	11 ± 0.97

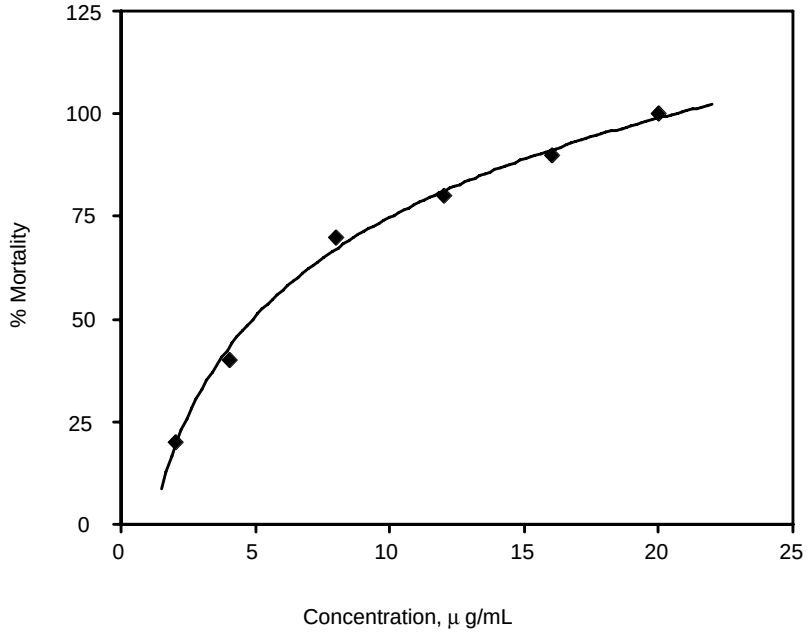
Results are shown in mean ± SEM and compared with normal (without EAC bearing) and control (EAC bearing mice) group. Number of mice/group was 4. * $p < 0.001$ when compared with control.

Table 4: Effect of Ni(II) complex on serum alkaline phosphatase activity of normal and tumor bearing mice.

Name of experiment	Enzyme activity (μmol of PNPP hydrolysed $min^{-1}mL^{-1}$ serum)	Enzyme activity %
Normal	$(28.69 \pm 0.71) \times 10^{-3}$	100.00
Control (only EAC bearing)	$(8.48 \pm 0.40) \times 10^{-3}$	29.56
EAC+Bleomycin (0.3 mg/Kg)	$(26.83 \pm 0.95) \times 10^{-3}$	93.35
EAC+Ni(II) complex (2 mg/Kg)	$(8.99 \pm 0.85) \times 10^{-3}$	31.33
EAC+Ni(II) complex (5 mg/Kg)	$(10.51 \pm 0.13) \times 10^{-3}$	36.63
EAC+Ni(II) complex (10 mg/Kg)	$(19.90 \pm 0.38) \times 10^{-3}$	69.36
Normal+Ni(II) complex (2mg/Kg)	$(23.95 \pm 0.41) \times 10^{-3}$	83.48
Normal+Ni(II) complex (5mg/Kg)	$(26.38 \pm 0.75) \times 10^{-3}$	91.95
Normal+Ni(II) complex (10mg/Kg)	$(28.18 \pm 0.70) \times 10^{-3}$	98.22

Number of mice per group was 4. Results are shown as mean ± SEM. * $p < 0.001$ when compared with control.

Figure 2: Brine shrimp lethality of Ni(II) cystine complex.



Statistical analysis

Significance of the experiments were statistically evaluated by students "t" test.

RESULTS AND DISCUSSION

Analysis of IR data

The formation of the Ni(II) complex has been supported from IR spectral data. The peak at 1630 cm^{-1} for ν_{COOH} was absent in the spectrum of the complex due to the bonding of carboxylate ion with the metal. Likewise due to the formation of Ni–N bond, peak for ν_{C-N} at 1390 cm^{-1} was also found to be absent in the spectrum. Further ν_{N-H} (1590 cm^{-1}) (in ligand) has been shifted to 1550 cm^{-1}. Peaks for ν_{C-S} (at 850 cm^{-1} and 790 cm^{-1}) and ν_{S-S} (at 480 cm^{-1} and 420 cm^{-1}) also shifted to down fields [840 and 770 cm^{-1} for ν_{C-S} and 400 cm^{-1} for ν_{S-S}] because of the formation of M–S bond. The presence of H_2O showed a broad peak at 2950 cm^{-1} to 3600 cm^{-1}. This broad peak was also coupled with ν_{NH_2}

However the peaks due to Ni–O, Ni–N and Ni–S could not be ascertained owing to the inability of getting clear indications at the lower part of the spectrum.

Effect of Ni(II) cystine complex on cell growth inhibition

Effect of Ni(II) complex and *bleomycin* on EAC cell growth on day 5 after tumor transplantation are shown in Table 1.

Treatment with Ni(II) complex resulted in pronounced cell growth inhibition at doses 10 mg/Kg (i.p) and 5 mg/Kg (i.p) as evident from 76.18% and 72.1% reduction of

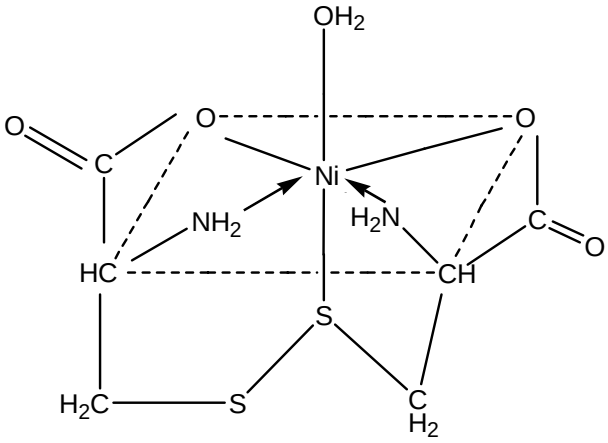


Table 5: Effect Ni(II) cystine complex on the enhancement of normal peritoneal cells of mice.

Name of experiment	Dose	Macrophase mean ± SEM	Total peritoneal
Normal	-	$(2.3 \pm 0.21) \times 10^6$	$(18.9 \pm 0.91) \times 10^6$
Normal + Ni(II) complex	5 mg/Kg	$(2.5 \pm 0.30) \times 10^6$	$(19.5 \pm 0.75) \times 10^6$
	10 mg/Kg	$(2.9 \pm 0.25) \times 10^6$	$(21.9 \pm 0.83) \times 10^6$

tumor cells respectively. However still lower doses (2 gm/Kg ip) could inhibit cell growth at reduced rate. In comparison 93.8% cell growth inhibition was found with *bleomycin* at dose 0.3 mg/kg.

Effect of Ni(II) complex on survival time

Effect of Ni(II) complex at different doses have been summarized in Table 2. It was found that treatment of the tumor induced test animals with the compound at the doses of 2 mg/kg, 5 mg/kg and 10 mg/g resulted in increase of life span by 8.38%, 19.20% and 44.72% respectively when compared to control. Thus the survival time was found to be increased when the dose of the compound was increased from 2 mg/kg to 10mg/kg. It was noticed that the *bleomycin* increases the life span by 82.82% when compared to control.

Although the increase of life span of EAC bearing mice with Ni(II) complex is not very promising, the cell growth inhibition rate on the contrary is quite encouraging. Ni(II) cystine complex at the doses above 5 mg/Kg is comparable to that of *bleomycin* (Table 1).

Effect of Ni(II) complex on tumor weight due to tumorogenesis in mice (Average tumor weight)

Effect of the test compound at doses 2mg/kg, 5mg/kg and 10mg/kg (i.p.) and the antibiotic *bleomycin* on the tumor weight due to tumorogenesis (average tumor weight) are shown in Figure 1. Treatment of the animals with the test compound, previously inoculated with EAC cells resulted in the inhibition of tumor growth. In case of the control (EAC) group, the body weight was increased by 50% in 20 days as compared with the normal. With Ni(II) complex at the doses of 2mg/Kg, 5mg/kg and 10 mg/kg; the body weight was found to be increased by 39.75%, 28.75% and 22.2% respectively. The reduction in rate of weight increase was obviously due to the inhibition of tumor growth as compared to that of normal mice during the same period.

Evidently the use of higher doses of the test compound fairly inhibited the tumor growth in test animals. This result is very much in accordance with the cell growth inhibition percentage obtained by *bleomycin.*

Effect of the Ni(II) complex on haematological parameters in normal tumor bearing mice on day 12 of tumor inoculation

The growth of tumor in mice induced by EAC cells affected in anemic condition as indicated by the significant decrease of the red blood cells and haemoglobin content determined on day 12 of tumor inoculation. When compared to normal test animals under similar condition (Table 3), it was found that red blood cell, haemoglobin percentage, lymphocyte value of EAC bearing mice were less than those of normal mice. That means when mice were attacked in cancer, red blood cells were decreased or decayed with the increase of white blood cells. After treatment with the test compound, it was found that the parameters restored slightly at the dose of 2mg/kg. But at the medium dose of 5mg/kg RBC, WBC, Hb% and differential counts restored to a greater extent. More significant changes were observed in counting monocytes. Obviously at still higher dose (10mg/kg), all these altered parameters were found to be recovered towards normal.

No significant effect on the haematological parameters of the test compound was found in case of normal mice.

Effect of the Ni(II) complex on serum alkaline phosphatase activity level in normal and tumor bearing mice on day 12 of tumor inoculation

Effect of the Ni(II) complex at the doses of 2 mg/kg, 5 mg/kg and 10 mg/kg on serum alkaline phosphatase on day 12 of tumor inoculation in normal and tumor bearing mice was summarized in Table 4. Serum alkaline phosphatase activity level in tumor bearing mice was markedly decreased by 70.44% due to tumorogenesis when compared to the normal mice. Treatment with the Ni(II) complex at the dose of 2mg/Kg restored the enzyme activity very insignificantly (1.77% only). Higher recovery by 7.07% and 39.80% was observed after treatment with higher doses at 5 mg/kg (i.p.) and 10 mg/kg (i.p.) respectively. Results were compared with *bleomycin* and are shown here (Table 4). In normal mice the Ni(II) cystine complex showed practically a little toxic effect on serum alkaline phosphatase activity.

Lethal dose (LD_{50}) value

The LD_{50} value was found to be 50 mg/Kg (i.p.), which represented the medium toxicity of the compound

Brine Shrimp Lethality bioassay

Ni (II) complex showed strong cytotoxic effect against brine shrimp nauplii. Median lethal concentration (LC50) of the test compound was determined and found to be 5.0 μg/mL (Figure 2).

Effect of Ni(II) complex on the enhancement of normal peritoneal cells of mice

Effect of Ni(II) complex on the enhancement of total peritoneal cells in normal mice are shown in the Table 5. Treatment with the Ni(II) complex at the doses of 5 mg/Kg and 10 mg/Kg showed some positive effects on enhancement of number of peritoneal cells. The number of macrophages are also increased to some extent. This enhancement (19) might have produced some cytokinetic products such as tumor necrosis factor, interleukim, interferons etc. which in turn may be responsible for killing the tumor cells.

In conclusion, Ni(II) cystine complex can be considered as a new compound, having fairly antineoplastic activity. Further experimental works should be carried out in an advanced level for obtaining more promising results.

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