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Antineoplastic activity of N–salicylideneglycinato–di–aquanickel(II) complex against Ehrlich Ascites Carcinoma (EAC) cells in mice

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ABSTRACT

In order to find out compounds having antineoplastic activities N-salicylideneglycinato-di-aquanickel(II) complex was synthesized and characterized. The antitumour activity was studied against Ehrlich Ascite Carcinoma (EAC) cells in Swiss Albino mice by monitoring the parameters like tumour weight measurement, survival time of tumour bearing mice, tumour cell growth inhibition etc. Some haematological parameters such as RBC, WBC, Hb%, differencial counts (lymphocytes, neutrophill, monocytes), alkaline phosphatase activity etc. were also measured. The results showed that Ni(II) complex has positive effect against EAC cells. This assessment was done by comparing the results with those obtained with the standard drug *bleomycin.* The compound can be considered as an effective anticancer agent.

Keywords: Antineoplastic activity, N-salicylideneglycinato-di-aquanickel(II) (SGN), EAC, and ALP.

INTRODUCTION

Many natural (Sur and Ganguly, 1994) and synthetic compounds (Pal et al., 1993) are capable of affecting selectively specific organs and tissues within biological systems. Among such, metal complexes (Pal et al., 1993; Daula et al., 2004 and Khanam et al., 2006) and specially those of schiff bases (Afrasiabi et al., 2005; Islam et al., 2002; Rodriuez-Arguelles et al., 2004 and Kasuga et al., 2001) are well known for their biological activities. Kasuga et al. (2001) studied the antimicrobial activities of Ni(II) complexes with thiosemicarbazone and semicarbazone as ligands. Recently, the antitumour activity of schiff base complexes of lanthanides (Yang et al., 2000) and also those of vanadium (Nobila et al., 2005) have been reported in the literature.

Realizing the possibilities of using such complexes as biologically active agents, N – salicylideneglycinato – di – aquanickel(II) abbreviated as SGN was prepared and used to study its antineoplastic activity. For the purpose a number of parameters viz. cell growth inhibition, average tumour weight, survival time of EAC cell bearing mice were studied. In addition the rectifying ability of depleted blood parameters and alkaline phosphatase activity of tumour bearing mice were also studied.

MATERIALS AND METHODS

Synthesis

The complex SGN was synthesized by the method described by Theriot et al. (1969). Solutions of glycine (0.7507 gm) in water and salicylaldehyde (1.22 gm) in alcohol were

mixed together (1:1 molar ratio) and refluxed for 10 hours. The solution obtained was then concentrated by distillation. Saturated solution of Ni(II) acetate (2.4874 gm in ethanol) was then mixed with the condensed solution. The resulting solution was refluxed again for 5–6 hours and cooled to room temperature. Greenish yellow crystals of Ni(II) complex precipitated. The crystals were washed several times with distilled water and finally with ethanol. The product was dried over silica gel and stored in a desiccator.

Characterization

Elemental analysis for CHN and metal of SGN were done by an elemental analyzer (Perkin 240C, USA) and an Atomic absorption spectrometer (Shimadzu, Japan) respectively. TGA thermogram was taken with a thermoanalyzer of Mettler Instrument Corp (Highstown, NJ).

Animal

Swiss Albino mice were collected from the International Center for Diarrheal Diseases Research, Bangladesh (ICDDR'B). For the experiment, male mice of 6–8 weeks of age, having weight 20±5 grams were used.

Tumour cells

Ehrlich Ascites Carcinoma (EAC) cells were obtained through courtesy of Indian Institute of Chemical biology (IICB), Kolkata, India. EAC cells were maintained every twelve days by intraperitoneal inoculation of 1.4×10^5 cells per mouse.

Determination of median lethal dose (LD_{50})

The lethal dose LD_{50} of the test compound was determined by recording the mortality after 24 hours of treatment following conventional method (Litehifield and Wilcoxon, 1949).

Cell growth inhibition

For this study, EAC cells (1.4×10^5) were inoculated into 5 groups of mice (6 in each) on day 0. Treatments were started after 24 hours of tumour inoculation and continued for 5 days. Group 1–3 received SGN at the dose of 2 mg/kg i.p, 5 mg/kg i.p, 10 mg/kg i.p per day per mouse respectively. Group 4 received *bleomycin* (0.3 mg/kg) and finally group 5 was considered as untreated control. Mice were sacrificed on the 6th day after transplantation and total tumour cells were harvested by repeated intraperitoneal wash with 0.9% saline. Viable cells per animal of the treated group were compared with those of the control group.

Average tumour weight and survival time

These parameters were measured (Sur et al., 1984) under similar experimental conditions as stated in the previous experiment "Cell growth inhibition" but here treatment was continued for 10 days. Tumour growth was monitored by recording daily weight changes and host survival time was recorded. The mean survival time in days and percent increase of life span were calculated as follows:

$$\text{MST} = \frac{\sum ST\ (days)\ of\ EMG}{Total\ number\ of\ mice}$$

where MST= mean survival time; ST = survival time; EMG = each mouse in a group.

$$\%\ \text{ILS} = \left[\frac{MST\ of\ treated\ group}{MST\ of\ control\ group} - 1\right] x100$$

where % ILS = Percent increase of life span.

Haematological study

To study the effect of the test compound on haematological parameters, 4 groups of mice (4 in each) were taken for both normal and EAC bearing mice, each treated with the test compound at the dose of 0, 2, 5 and 10 mg/kg i.p. respectively. Treatment was continued for 10 consecutive days. Blood was collected from each mouse on day 12 and the total count of white blood cells (WBC) and red blood cells (RBC) as well as haemoglobin content were determined by standard methods (Mukherjee, 1988) using the specific cell counting fluid and a haemocytometer. The differential count was carried out with wright stain.

Alkaline phosphatase activity (ALP)

ALP activity of the serum of normal and tumour bearing mice treated with the complex (2 mg/kg i.p, 5 mg/kg i.p and 10 mg/kg i.p 10 days) was assayed on day 12. The ALP activity was measured according to the procedure of Telfer (1993) using

paranitrophenyl phosphate (PNPP) as substrate in glycine sodium hydroxide buffer (pH 10). Absorbance was measured at 410 nm.

Brine shrimp lethality bioassay

This was done according to the method described by Atta-ur-rahman et al. (1999). Clean 6 vials were taken for the samples of five concentrations and the sixth one for control test. Exactly 5 mL of seawater (3.8% NaCl solution) was poured into each of the vials. Then with the help of a micropipette specific volumes of sample were transferred from stock solution to the vials to get final sample concentrations of 10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL and 100 µg/mL. With the help of a Pasteur pipette, living shrimps were taken to each sample solution and also to the control vial. After 24 hours vials were observed and the number of survived nauplii in each vial was counted.

Statistical analysis

Student t-test was used for the statistical analysis of the results. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The elemental analysis data for the test compound presented here were the average of three replications along with the theoretical values within parenthesis (Table 1). Since the experimental data are quite similar to those of the theoretical values, the synthesized compound can be taken as N-salicylideneglycinato-di-aquanickel(II). This is further supported from the TGA thermogram data for the decrease in weight due to the loss of coordinated water at 120–140 ^{o}C. The melting point of the compound could not be measured because of its decomposition at above 180 ^{o}C. It is to be noted here that, Theriot (1969) et al. synthesized this compound and proposed a dimeric structure for it (Figure 1). This structure is quite in accordance with the data obtained during this work. The IR spectral data were also consistent with the proposed form.

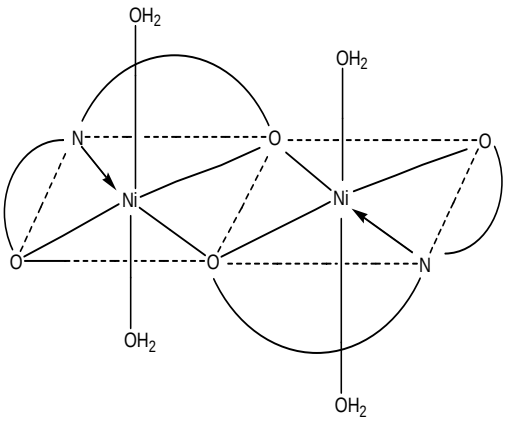
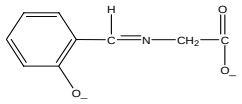


Figure 1: Structure of SGN dimmer complex, $[Ni\ L\ 2H_2O]_2$.

Where L =



The peak due to ν_{C-N} decreased from 1334 cm^{-1} (for glycine) to 1307 cm^{-1} indicated indirectly the formation of Ni–N bond. This bond formation was also supported from the shifting of $\nu_{C=N}$ peak 1690 cm^{-1} (schiff base) to 1640 cm^{-1}. But this shift was found to lie in the region having a number of peaks (1645, 1625, 1600, 1550 cm^{-1}). These additional peaks might have appeared from the absorption of aromatic rings and also from the lowering of asymmetric vibration ν_{COO-} (~1700 cm^{-1}) to down field most probably due to the formation of Ni–O bonds. The lowering of peak for phenolic ν_{OH-} (~3600 cm^{-1}) to down field appeared in the region 3600–3400 cm^{-1} which was again coupled with the absorption of coordinated water molecules.

The LD_{50} value of SGN complex was found to be 55 mg/kg i.p. (Table 2). The

Table 1: Elemental analysis data for the compound, SGN.

Percentage of elements present in the test compound (SGN)			
C ± SD	**H ± SD**	**N ± SD**	**Ni ± SD**
39.17 ± 0.27	4.01 ± 0.049	5.26 ± 0.14	20.96 ± 0.118
(39.75)	(4.05)	(5.10)	(21.60)

Values = experimentally found; (values) = theoretically calculated; SD = Standard deviation.

antineoplastic activity was studied by monitoring the parameters, 'cell growth inhibition' and 'survival time' of tumour bearing mice. The tumour cell growth inhibition ability of the test compound was found to be very promising. This ability increased remarkably even with the low dose (2 mg/kg i.p). Above 10 mg/kg i.p the activity of the test compound can be compared with that of *bleomycin* at 0.3 mg/kg i.p (Table 3). *In vivo,* the mean survival time (MST) for untreated tumour bearing mice was 21.2 days. Treatment with the Ni(II) complex increased the value remarkably. About 70% enhancement of MST was found after treatment at dose 10 mg/kg (i.p). *Bleomycin* at the dose 0.3 mg/kg (i.p) increased this value to 90% (Table 4).

The haematological parameters were examined in normal, EAC cell bearing and test compound treated mice. In EAC cell bearing mice (after 12 days) all the parameters such as haemoglobin, WBC, RBC and differential counts (monocytes, lymphocytes, neutrophils) significantly changed as compared to those of the normal mice. After treatment with the test compound at dose 10 mg/kg (i.p) these parameters were found to be restored towards normal values. However, the results obtained with the test compound at lower doses below 10 mg/kg i.p were not found to be very significant (Table 5).

The host toxic effect of the test compound on normal mice was also determined. After treatment with the test compound at dose 5 mg/kg for 10 consecutive days, the haematological parameters (RBC, haemoglobin, monocytes, etc.) were found to decrease gradually from normal values. But after 12 days lymphocytes and neutrophil values restored significantly (Table 5).

In tumour bearing mice, the serum alkaline phosphatase (ALP) activity decreased with the simultaneous increase of tumour growth. After treatment with the test compound increase of ALP activity with the reduction of tumour growth were observed. Significant recovery of ALP activity towards the normal value was achieved again with the dose of 10 mg/kg of the Ni(II) complex (Table 6).

The Ni(II) complex showed a prominent effect on Brine Shrimp lethality bioassay with LC_{50} 28.3 µg/mL (Figure 2). The positive response suggested that Ni(II) complex might have biological activities.

Table 2: Data for LD_{50} determination with SGN complex.

Dose, mg/kg	35	45	50	55	60	65
Mortality %	10	20	40	50	70	90

No. of mice per group: 10; Recording time: 24 hours after treatment; SGN = N- salicylideneglycinato-di-aquanickel(II).

Table 3: Effect of SGN complex on EAC cell growth inhibition (*in vivo*).

Test compounds	Nature of the drug	Dose (mg/kg)	Number of EAC cells/mice on day 5 after tumour cell inoculation	% of cell growth inhibition
Control (EAC) bearing mice	-	-	$(17.08\pm1.12) \times 10^7$	-
Bleomycin	Antibiotic	0.3 mg/kg	$(2.01\pm 0.25) \times 10^7$	88.23 P = 0.01
SGN–complex	Synthetic	2 mg/kg	$(7.33\pm0.09) \times 10^7$	57.07 P = 0.1
	Synthetic	5 mg/kg	$(3.36\pm0.53) \times 10^7$	80.34 P = 0.5
	Synthetic	10 mg/kg	$(2.63\pm0.11) \times 10^7$	84.59 P = 0.01

No. of mice per group were 6; Values represent mean ± SEM; P-probability function; $P < 0.05$ is significant.
EAC = Ehrlich Ascites Carcinoma; SGN = N- salicylideneglycinato-di-aquanickel(II).

Table 4: Effect of test compound on survival time of EAC cell bearing mice.

Test compounds	Nature of drug	Dose (mg/kg)	Mean survival time (days), Mean ± SEM	% increase of life span
Control (only EAC bearing mice)	–	–	21.20 ± 4.31	–
EAC + *Bleomycin*	Antibiotic	0.3	40.00 ± 5.10	90.47 P < 0.01
EAC + Ni(II) complex	Synthetic	2.0	26.60 ± 3.32	25.47
	Synthetic	5.0	30.60 ± 2.15	44.34 P < 0.05
	Synthetic	10.0	36.20 ± 2.75	70.75 P < 0.01

No. of mice per group were 6; P- Probability function; SEM - Standard error of mean. EAC = Ehrlich Ascites Carcinoma;

Table 5: Effect of SGN complex on haematological parameters of normal and tumour bearing mice on day 12 of tumour inoculation.

Test compound	RBC cells/mL	WBC cells/mL	% Hb	Lymphocytes %	Neutrophil %	Monocytes %
Normal (N)	$(5.6\pm0.3)\times10^9$	$(10\pm0.7)\times10^6$	75.5±1.5	71 ± 1.06	25 ± 1.4	7 ± 0.7
Control (EAC)	$(2.3\pm0.2)\times10^9$	$(25.7\pm0.4)\times10^6$	45.0±1.2	54 ± 0.65	36 ± 1.2	9 ± 0.6
EAC + 2 mg/kg	$(2.5\pm0.1)\times10^9$	$(22\pm0.5)\times10^6$	46.0±0.4	69 ± 1.06	27 ± 1.1	6 ± 0.8
EAC + 5 mg/kg	$(3.6\pm1.4)\times10^9$	$(17.7\pm1.0)\times10^6$	51.3±1.0	71 ± 1.52	24 ± 0.5	4 ± 0.9
EAC + 10 mg/kg	$(4.4\pm0.8)\times10^9$	$(13.7\pm0.6)\times10^6$	53.4±0.6	73 ± 0.58	28 ± 1.0	1 ± 0.5
N+ 2 mg/kg	$(2.4\pm0.2)\times10^9$	$(7.8\pm0.7)\times10^6$	51.5±0.6	75 ± 0.45	21 ± 1.2	5 ± 0.5
N+ 5 mg/kg	$(2.1\pm0.1)\times10^9$	$(9.0\pm0.3)\times10^6$	44.2±1.6	73 ± 0.64	24 ± 0.5	3 ± 0.4
N+ 10 mg/kg	$(1.9\pm0.3)\times10^9$	$(9.3\pm0.3)\times10^6$	41.3±1.4	72 ± 0.71	26 ± 1.0	2 ± 0.3

Number of mice were 4 per group. Results are shown as mean ± SEM and compared with normal (mice without EAC cell) and control (EAC bearing mice). EAC = Ehrlich Ascites Carcinoma; SGN = N- salicylideneglycinato-di-aquanickel(II).

Table 6: Effect of SGN complex on serum alkaline phosphatase activity in normal and tumour bearing mice.

Name of experiment	Enzyme activity of (µmol of PNPP hydrolysed min^{-1} mL^{-1} of serum)
Normal	$(18.41 \pm 0.56) \times 10^{-3}$
Control (only EAC cell bearing mice)	$(7.19 \pm 0.22) \times 10^{-3}$
DMSO	$(16.65 \pm 0.47) \times 10^{-3}$
EAC + *Bleomycin* (0.3 mg/kg)	$(15.47 \pm 0.41) \times 10^{-3}$ ($p<0.01$)
EAC + Test compound (2 mg/kg)	$(8.52 \pm 0.65) \times 10^{-3}$ ($p<0.5$)
EAC + Test compound (5 mg/kg)	$(12.81 \pm 0.82) \times 10^{-3}$ ($p<0.05$)
EAC + Test compound (10 mg/kg)	$(18.54 \pm 0.75) \times 10^{-3}$ ($p<0.01$)
Normal + Test compound (2 mg/kg)	$(17.83 \pm 1.32) \times 10^{-3}$ ($p<0.01$)
Normal + Test compound (5 mg/kg)	$(17.98 \pm 0.16) \times 10^{-3}$ ($p<0.001$)
Normal +Test compound (10 mg/kg)	$(18.36 \pm 1.92) \times 10^{-3}$ ($p<0.01$)

Number of mice per group were 4. Results are shown as mean ±SEM. P-Probability function; P < 0.05 is statistically significant. SEM - Standard error of mean. EAC = Ehrlich Ascites Carcinoma; SGN = N- salicylideneglycinato-di-aquanickel(II).

Conclusion

The dose 10 mg/kg i.p. of SGN was found to be quite suitable for performing such experiments. Since the LD_{50} value was found to be much higher than the doses used in the experiments, the compound, SGN can be considered as an effective anticancer drug. However this conclusive statement should be confirmed after performing further experiments with different cell lines using more advanced technologies.

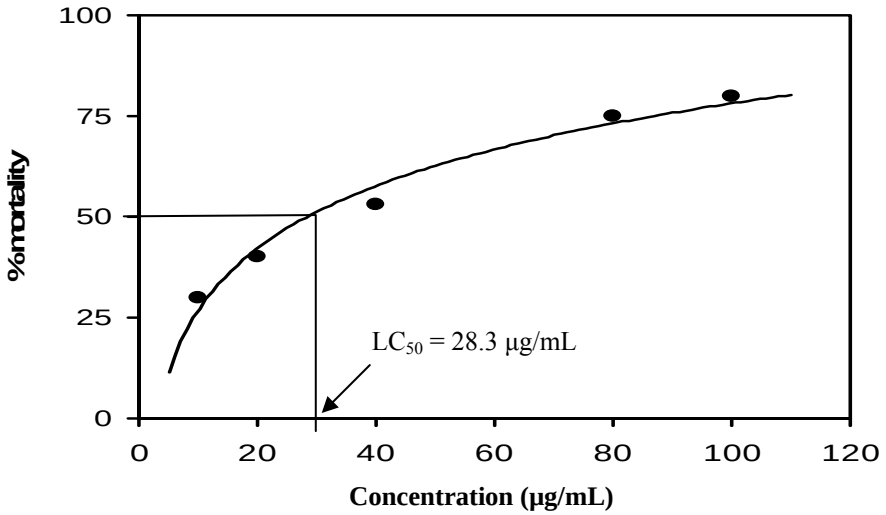


Figure 2: Brine shrimp lethality bioassay of SGN complex.
SGN = N- salicylideneglycinato-di-aquanickel(II).

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