In Vivo Anticancer Activities of Vanillin Thiosemicarbazone Complexes with Co(II) and Ni(II)

Sha M. S. SHAHRIAR[1], S. M. M. ALI[1], M. JESMIN[1]

[1]Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Rajshahi-6205, Bangladesh.

ABSTRACT

The anticancer activities of vanillin thiosemicarbazone complexes with Co(II) and Ni(II) have been studied against Ehrlich ascites carcinoma (EAC) cells in Swiss albino mice. Both the complexes were administrated into the intraperitoneal cavity of the EAC-inoculated mice at two doses (5 and 7.5 mg/kg i.p.). The anticancer activities were studied by monitoring the parameters such as cell growth inhibition, tumor weight measurement, survival time of EAC-bearing mice, as well as the changes in depleted hematological parameters due to tumorigenesis. All data were compared with those of a known standard drug bleomycin at the dose of 0.3 mg/kg (i.p.). It has been found that these complexes significantly increased the life span of tumor-bearing mice and decreased the rate of growth and weight of tumor cells. They also restored modestly the depleted hematological parameters such as hemoglobin content, RBC count, and WBC count to normal. They enhanced the number of macrophages in normal mice with minor host toxicity. It is concluded that these compounds can be primarily considered as potent anticancer agents.

Key words: anticancer activity, EAC cells, hematological parameters, macrophages

INTRODUCTION

Schiff bases are the most widely studied biologically active compounds nowadays. Schiff bases alone and their metal complexes have a wide range of anticancer, antibacterial, antifungal, anti-inflammatory, antitubercular, analgesic, and pesticidal activities(1-8).

Among the Schiff bases, the semicarbazones (9-11) are found to be potent anticancer agents. Recently the studies have been extended with thiosemicarbazones (12). Not only the Schiff bases, but Schiff base complexes (4,13) with transition metals have also been investigated against Ehrlich ascites carcinoma (EAC) cells. In the present study, vanillin thiosemicarbazone complexes with Co(II) and Ni(II) were selected as test compounds and their anticancer activities against EAC cells in vivo were studied. Hematological studies were also conducted in support of this work.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used to carry out the research work were of reagent grade.

Experimental animals

Swiss albino mice, 5-7 weeks old, and weighing 20-26 g, were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B), Mohakhali, Dhaka.

Correspondence:
Sha M. S. Shahriar
Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Rajshahi-6205, Bangladesh.
e-mail: shahan.acrubd@yahoo.com

Table 1: Physical constants of the synthesized compounds

Compound	LD_{50}, mg/kg	Melting point, °C	Color	Solubility	IR data (s for strong, m for medium)
$Co(VTSC)_2$	60	> 300°C	Black	DMSO, Ethanol	3150s, 1620s, 1580s, 660m, 550m
$Ni(VTSC)_2$	80	236–240°C	Grey	DMSO, Ethanol	1350m, 1610m, 1585s, 660m, 550m

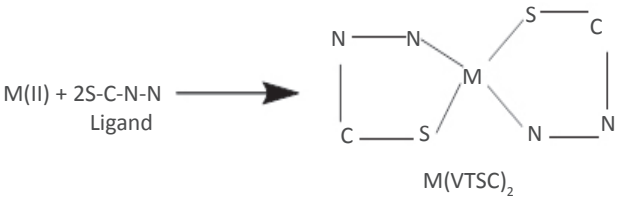
Animal care

The mice were kept in iron cages with saw dust and straw bedding, which were changed once a week regularly. Standard mouse diet (recommended and prepared by ICDDR'B) and water were given adequately.

Synthesis of Schiff base complexes

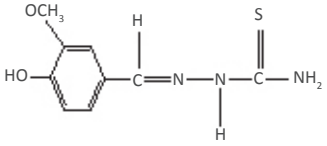
The complexes of Co(II) and Ni(II) were prepared (14) by adding a hot aqueous solution of the respective metal acetate drop-wise to a refluxing methanolic solution of the ligand containing sodium acetate (1 g) until the metal to ligand ratio reached 1:2. The solid separated was filtered, washed with water and then with methanol, and dried over P4O10. The formation of the synthesized compounds was verified by measuring melting points and conducting infrared spectral studies (14). The disappearance of peaks at 775 cm^{-1} (for γc=s) and appearance at 660 cm^{-1} (for γc-s) confirmed the formation of M–S bond in the complexes. Furthermore, the appearance of peak at 550 cm^{-1} in both the complexes might be related to M-N where M stand for metal viz. Co(II) or Ni(II), which is given bellow. The shifting of the peak for C=N (at 1620 cm^{-1}) to a lower wave number (~ 1580 cm^{-1}) supported the participation of N (of the ligand) in the complex formation.

The reactions and the structure (square planar) are given below.



$M(VTSC)_2$

where ligand S-C-N-N stands for the ligand.



and M for Co(II) and Ni(II).

Determination of median lethal dose (LD_{50})

The test compounds were separately dissolved in 2% dimethyl sulfoxide and injected intraperitoneally into six groups of mice (each group containing six animals) with different doses. The LD_{50} values were estimated(15) from the plots of mortality versus dose curve.

Characteristics

The physical constants of the synthesized compounds are presented in Table 1.

Tumor cells

Transplantable tumor (EAC) cells were used in this experiment. The initial inoculums of EAC cells were kindly provided by the Indian Institute of Chemical Biology (IICB), Kolkata, India. The EAC cells were thereafter propagated in our laboratory biweekly through intraperitoneal (i.p.) injections of 2×10^6 cells per mouse.

Study of anticancer activities

The procedure followed for determining the anticancer activities of the compounds was similar to that described in the recently published papers(9).

Cell growth inhibition

Six groups of mice (six in each group) were used for the experiment. In every mouse 2×10^6 EAC cells were inoculated into each group on day 0. Treatments were started after 24 h of tumor inoculation and continued for 5 days. Groups 1 and 2 received the Ni(VTSC)2 at the doses of 5 and 7.5 mg/kg (i.p.) respectively, and groups 3 and 4 were treated with Co(VTSC)2 at the same dose. Group 5 received bleomycin at the dose of 0.3 mg/kg (i.p.), and group 6 was used as control. Mice in each group were sacrificed on day 6, and the total intraperitoneal tumor cells were harvested using normal saline (0.98%). Viable cells were first identified using trypan blue and then counted using a

hemocytometer. The total number of viable cells in every animal of the treated groups was compared with that of the control (EAC treated only) group.

Average tumor weight and mean survival time (MST)

Six groups of mice (six in each group) were used for this experiment. Groups 1 and 2 were treated with Ni(VTSC)2 and groups 3 and 4 with Co(VTSC)2 at the doses of 5 mg/kg and 7.5 mg/kg, respectively. Group 5 received bleomycin at the dose of 0.3 mg/kg. Group 6 served as control. EAC cells (2×10^6) were inoculated in each mouse on day 0. Treatment was started after 24 h of tumor cell inoculation and continued for 10 days. The weight changes of each mouse were recorded daily, and the increase in tumor weight was monitored. The host survival was recorded and expressed as mean of survival time in days. The percent increase in life span (ILS) was calculated by using the following formulae:

MST = ∑Survival time (days) of each mouse in a group/ Total number of mice

ILS% = (MST of treated group/MST of the control group − 1) × 100

Hematological parameters in normal and tumor-bearing mice

The effect of the test compounds on hematological parameters was studied in both normal and tumor-bearing mice. For tumor-bearing mice, treatment was started after 24 h of EAC cell transplantation and continued for 10 days. The treatment schedule was the same as above. For normal mice (free from EAC cells), the same procedure was followed. Blood was drawn out (from tail vain) from each group of mice on day 5, 10, 15, and 25 for such studies.

Effect of the test compounds on normal peritoneal cells

The effects of the test compounds on normal peritoneal cells were measured by counting total peritoneal cells and macrophages. Five groups of normal mice (four in each group) were used for this test. The treatment was continued for three consecutive days. Groups 1 and 2 received Ni(VTSC)2 and groups 3 and 4 received Co(VTSC)2 at doses of 5 mg/kg and 7.5 mg/kg, respectively. The untreated group (group 5) was used as control. On the fourth day, the animals were sacrificed after injecting 5 mL of normal saline (0.98%) into the peritoneal cavity of each mouse. Intraperitoneal exuded cells and macrophages were counted with 1% neutral red using the hemocytometer.

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's t test using SPSS statistical software (SPPSS: An IBM Company, IBM Corporation 1 New Orchard Road Armonk, New York 10504, 1722, United States 914-499-1900). version 14. $P < 0.05$ was considered to be statistically significant compared with the control.

RESULTS

In most cases, average values of repeated experiments were taken. The lethal doses of Co(II) and Ni(II) complexes were found to be 60 mg/kg and 80 mg/kg for intraperitoneal treatment in Swiss albino mice. The effects of the test compounds and bleomycin on the tumor weight due to tumorigenesis are shown in Figure 1. The treatment of the animals with the test compounds, previously inoculated with EAC cells, resulted in the inhibition of tumor growth pronouncedly. The effects of test compounds and bleomycin on EAC cell growth on day 6 after tumor transplantation are shown in Table 2. On treatment with Co(VTSC)2 and Ni(VTSC)2, the cell growth inhibition at the dose of 7.5 mg/kg was found to be 71.08% and 86.85%, respectively. Bleomycin at the dose of 0.3 mg/kg (i.p.) showed 88.50% cell growth inhibition.

The effects of test compounds on the survival time at different doses are summarized in Table 3. It was observed that the life span increased significantly after treatment with the test compounds. On treatment with Co(VTSC)2 and Ni(VTSC)2 at the dose of 7.5 mg/kg, the life span increased by 58.57% and 83.81%, respectively, compared with the control mice. The survival time was found to be increased with increased doses. Bleomycin at the dose of 0.3 mg/kg (i.p.) increased the life span by 90.48% compared with the control.

The effects of the test compounds on the hematological parameters of the tumor-bearing mice are shown in Figures 2-4. These parameters varied from their normal values along with tumor growth. The hemoglobin content and RBC counts decreased, whereas the WBC counts increased after the inoculation of EAC cells. After treatment with the compounds, the parameters were restored moderately.

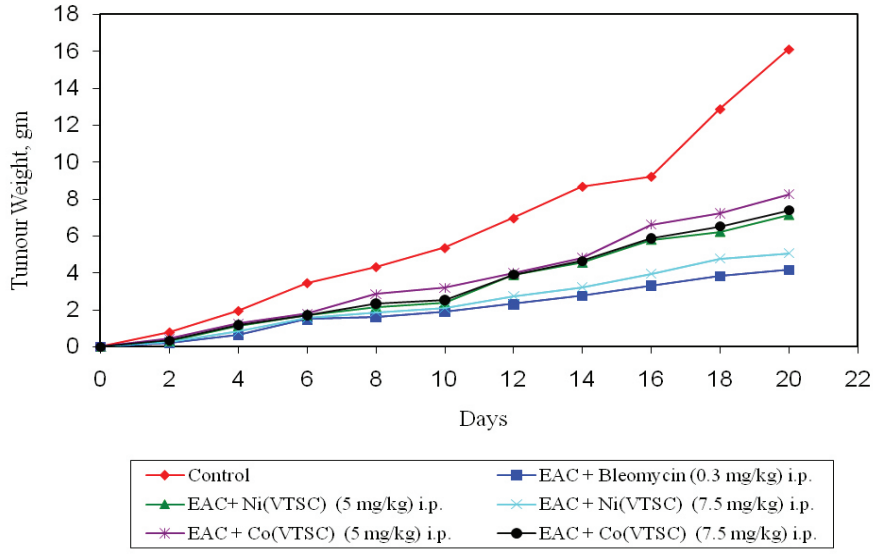


FIGURE 1: Effect of the test compounds on average tumor weight in mice (mean ± SEM) (n = 6).

TABLE 2: Effect of the test compounds and bleomycin on EAC cell growth inhibition (in vivo) (mean ± SEM) (n = 6).

Test compounds	Nature of the drug	LD_{50} (mg/kg)	Dose (mg/kg)	No. of EAC cells in mice on day 6 after tumor cell inoculation	Cell growth inhibition (%)
Control	-	-	-	$(6.275 \pm 0.034) \times 10^7$	-
Bleomycin	Antibiotic	128	0.3	$(0.721 \pm 0.005) \times 10^{7***}$	88.50
$Co(VTSC)_2$	Synthetic	60	5	$(2.619 \pm 0.08) \times 10^{7**}$	58.26
			7.5	$(1.815 \pm 0.006) \times 10^{7***}$	71.08
$Ni(VTSC)_2$	Synthetic	80	5	$(1.63 \pm 0.010) \times 10^{7***}$	74.02
			7.5	$(0.825 \pm 0.007) \times 10^{7***}$	86.85

Treatment days=5; **P < 0.01 and ***P < 0.001 between the control group and the treated group.

TABLE 3: Effect of the test compounds and bleomycin on survival time of EAC cell-bearing mice (mean ± SEM) (n = 6).

Test compounds	Nature of the drug	Dose (mg/kg) (i.p.)	Mean survival time (days)	Increase in life span (%)
Control	-	-	21.0 ± 0.78	-
Bleomycin	Antibiotic	0.3	40.0 ± 0.64***	90.48
$Co(VTSC)_2$	Synthetic	5	30.0 ± 0.65*	42.86
		7.5	33.3 ± 0.78**	58.57
$Ni(VTSC)_2$	Synthetic	5	33.8 ± 0.92**	60.95
		7.5	38.6 ± 1.10***	83.81

Treatment days=10; *P < 0.05, **P < 0.01, and ***P < 0.001 between the control group and the treated group.

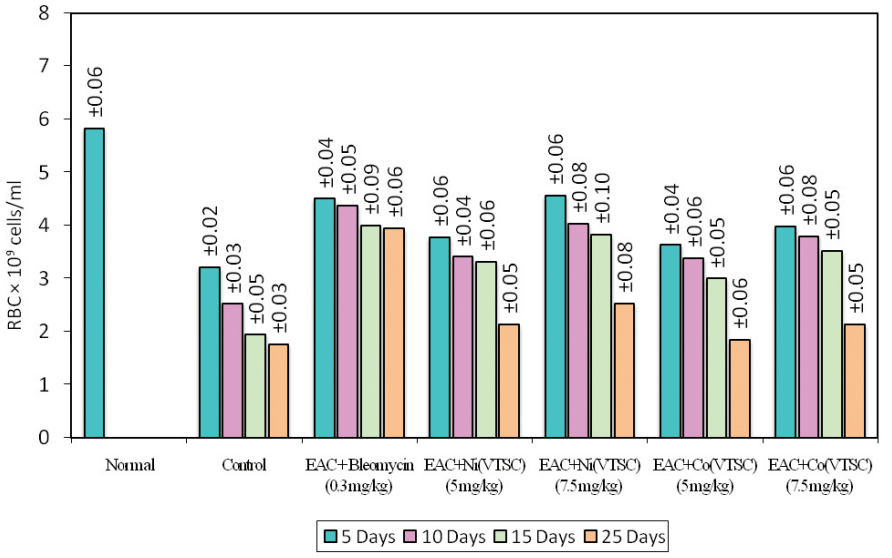


FIGURE 2: Effect of the test compounds on RBC count of EAC-bearing mice on days 5, 10, 15, and 25. Treatment days=10 (mean ± SEM) (n = 6).

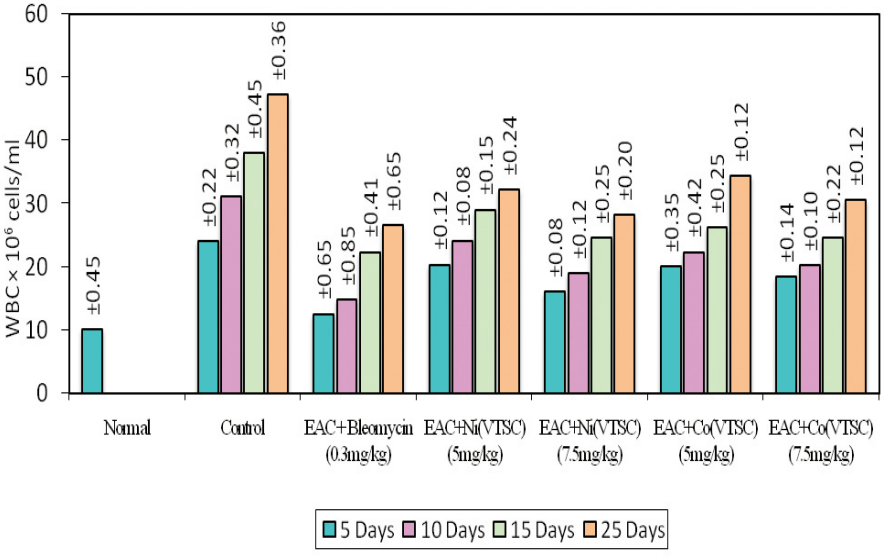


FIGURE 3: Effect of the test compounds on WBC count of EAC-bearing mice on days 5, 10, 15, and 25. Treatment days=10 (mean ± SEM) (n = 6).

The effects of the test compounds on the hematological parameters of the normal mice are shown in Figures 5–7. The test compounds showed low toxicity during the treatment period, but these parameters were almost restored back to normal values within 25 days.

The effects of the test compounds on peritoneal cells in normal mice at different doses are shown in Table 4. These bases noticeably enhanced the number of peritoneal macrophages.

DISCUSSION

The potency of the compound as an anticancer agent was judged by measuring (i) the reduction of average tumor weight, (ii) cell growth inhibition, and (iii) enhancement of life span of the EAC cell-bearing mice. The efficiency of the compound was compared with the data obtained by running parallel experiments with a known effective anticancer drug bleomycin at the dose of 0.3 mg/kg (i.p.) and also with those obtained with similar type of compounds available in the literature (10-11).

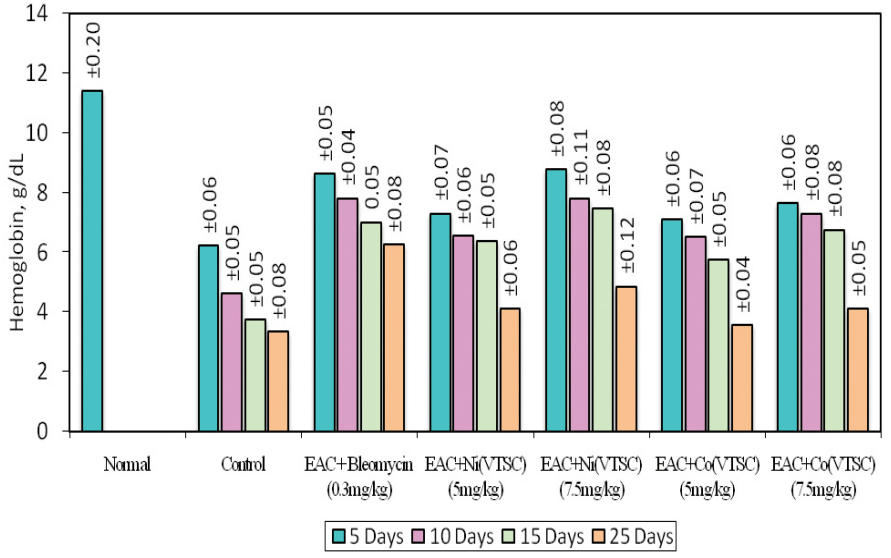


FIGURE 4: Effect of the test compounds on the hemoglobin content of EAC-bearing mice on days 5, 10, 15, and 25. Treatment days=10 (mean ± SEM) (n = 6).

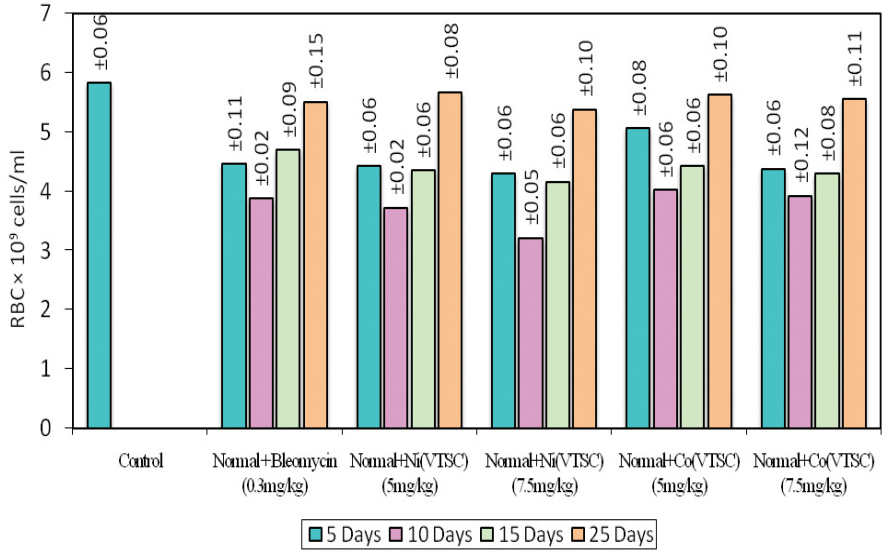


FIGURE 5: Effect of the test compounds on RBC count of normal mice on days 5, 10, 15, and 25. Treatment days=10 (mean ± SEM) (n = 6).

For EAC cell-bearing mice, the tumor weight was found to increase rapidly. The treatment of such mice with test compounds reduced the cell growth rate. The life span of the EAC cell-bearing mice increased remarkably when treated with the test compounds. The data were also comparable to those found in the literature (15). The prolongation of the life span of tumor-bearing mice is a very important and reliable criterion for judging the potency of any drug as an anticancer agent (16). In effect, the results were quite comparable to those obtained with other Schiff base complexes and also with bleomycin (4, 13). The positive effect of the compounds against EAC cell-bearing mice was further verified by monitoring the change in hematological and biological parameters. The RBC count and hemoglobin contents of EAC cell-bearing mice decreased gradually with time compared with those of normal mice. The reduction in both RBC count and hemoglobin content is the major problem in cancer-bearing animals. The problems that are usually encountered in cancer chemotherapy are myelosuppression and anemia. This is probably owing to the deficiency of iron in hemolytic or myelopathic conditions (17, 18). Treatment with the test compounds probably restored the RBC

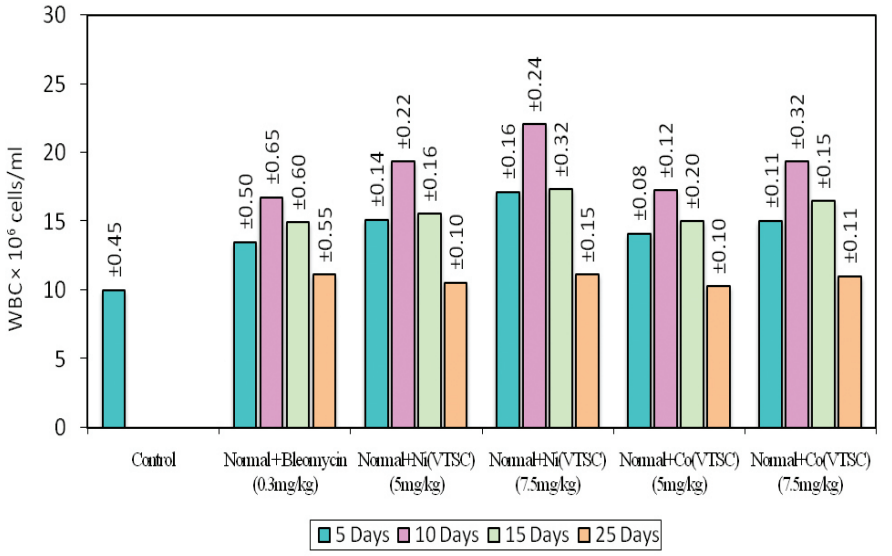


FIGURE 6: Effect of the test compounds on WBC count of normal mice on days 5, 10, 15, and 25. Treatment days=10 (mean ± SEM) (n = 6).

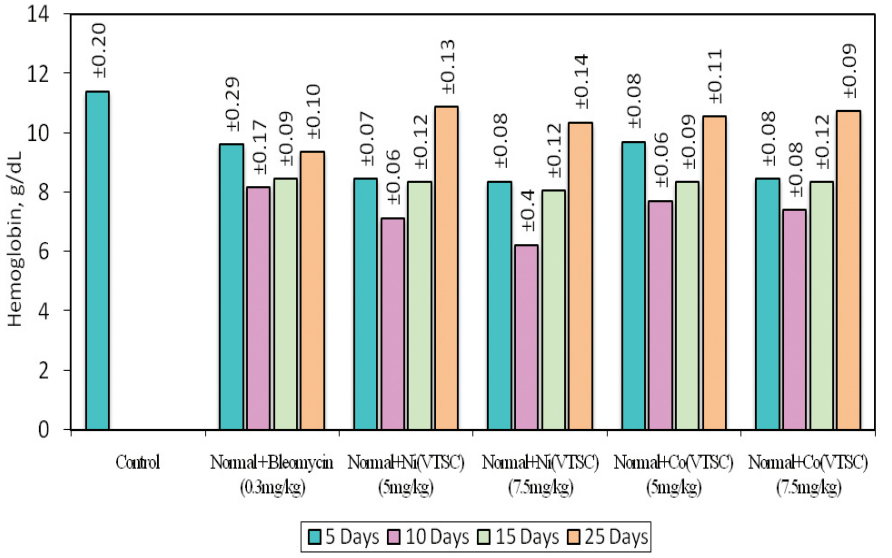


FIGURE7: Effect of the test compounds on the hemoglobin content of normal mice on days 5, 10, 15, and 25. Treatment days=10 (mean ± SEM) (n = 6).

count and hemoglobin content to normal, as the reduction slowed down compared with that in the EAC-bearing untreated mice. As the tumors grew, the WBC count increased with time. The rise in WBC count of the EAC-bearing treated mice followed a much slower rate compared with that of the EAC-bearing untreated mice. Parallel hematological experiments were conducted with normal mice to evaluate the host toxicity effect of the test compounds. A very slight deterioration of these parameters was observed during the treatment period (25 consecutive days). A similar toxic effect has also been observed (15) with bleomycin at the dose of 0.3 mg/kg (i.p.).

The immunological effect of the test compounds in fresh healthy mice was evaluated by counting peritoneal macrophages, which further supported the potency of the test compounds as anticancer agents. The test compounds noticeably enhanced the number of macrophages. This enhancement might have produced some cytokinetic products, such as tumor necrosis factor, interleukins, and interferons, which, in turn, might be responsible (19) for destroying the tumor cells.

Table 4: Effect of the test compounds on the enhancement of normal peritoneal cells in mice (mean ± SEM) (n = 6)

Treatment	Dose (mg/kg) (i.p.)	Macrophages (per mL)	Total peritoneal cells × 10^6 (per mL)
Control (normal)	-	1.65 ± 0.26	9.25 ± 0.32
Normal + $Co(VTSC)_2$	5	1.75 ± 0.11**	9.40 ± 0.16**
	7.5	1.86 ± 0.18***	9.62 ± 0.09***
Normal+ $Ni(VTSC)_2$	5	1.77 ± 0.20***	9.38 ± 0.12**
	7.5	2.21 ± 0.15*	9.75 ± 0.21*

Treatment days=3; *P < 0.05, **P < 0.01, and ***P < 0.001 between the control group and the treated group.

In conclusion, the test compounds showed pronounced efficiency against EAC cells in Swiss albino mice. Important characteristics such as cell growth inhibition, tumor weight reduction, and the enhancement of survival time of EAC cell-bearing mice with test compounds. The results were quite comparable to those obtained with other Schiff base complexes and bleomycin. The data may be valuable for advanced cancer researches in future.

CONFLICT OF INTEREST STATEMENT

We, the authors, declare no conflict of interest.

ACKNOWLEDGMENTS

The authors are thankful to the IICB, Kolkata, India, for providing the EAC cells. This work was supported by a grant from the National Science and Information and Communication Technology of Bangladesh [grant No. 39.012.002.01.03.015.2011-271(64)].

REFERENCES

1. Venkatesh P. Synthesis, characterization and antimicrobial activity of various schiff base complexes of Zn (II) and Cu (II) ions. Asian J Pharm Hea Sci 2011; 1: 8-11.
2. Ali MM, Jesmin M, Azad MAK, Islam MK, Zahan R. Anti-inflammatory and analgesic activity of acetophenone and benzophenone semicarbazones. Asian Pac J Trop Biomed 2012; 1-4.
3. Ali MM, Jesmin M, Salam SMA, Khanam JA, Islam MF, Islam MN. Pesticidal activities of some schiff bases derived from benzoin, salicylaldehyde, aminophenol and 2,4-dinitro phenyl hydrazine. J Sci Res 2009; 1: 641-646.
4. Ali MM, Jesmin M, Islam MN, Shahriar SMS, Habib MR, Islam MF, Khanam JA. Anticancer activities of some transition metal complexes of a schiff base derived from salicylaldehyde, glycine. ACGC Chem Res Comm 2009; 23: 13-22.
5. Jesmin M, Ali MM, Khanam JA. Antineoplastic activity of some schiff bases derived from benzoin, salicylaldehyde, aminophenol and 2, 4-dinitrophenyl hydrazine. Thai J Pharm Sci 2010; 34: 20-31.
6. Islam MN, Shahriar SMS, Islam MK, Jesmin M, Ali MM, Khanam JA. Antibacterial activities of some transition metal schiff base complexes. Int Letter Chem Phy Astro 2013; 5:12-20.
7. Sathe BS, Jaychandran E, Jagtap VA, Sreenivasa GM. Synthesis, characterization and anti-inflammatory evaluation of new fluorobenzothiazole schiff bases. Int J Pharm Res Dev 2011; 3: 164-169.
8. Pandey A, Dewangan D, Verma S, Mishra A, Dulbey RD. Synthesis of schiff bases of 2-amino-5-aryl-1,3,4-thiadizole and its analgesic, anti-inflammatory, antibacterial and antitubercular activity. Int J Chem Tech Res 2011; 3: 178-184.
9. Khanam JA, Islam MF, Jesmin M, Ali MM. Antineoplastic activity of acetone semicarbazone (ASC) against ehrlich ascites carcinoma (EAC) bearing mice. J Natl Sci Found 2010; 38: 225-231.
10. Ali MM, Azad MAK, Jesmin M, Ahsan S, Rahman M, Khanam JA, Islam MN, Shahriar SMS. In vivo anticancer activity of vanillin semicarbazone. Asian Pac J Trop Biomed 2012; 3: 438-442.
11. Islam MK, Ali MM, Jesmin M, Khanam JA. In vivo anticancer activities of benzophenone semicarbazone against ehrlich ascites carcinoma (EAC) cells in swiss albino mice. J Can Biol and Medi 2012; 9: 242-247.
12. Ali MM, Jesmin M, Islam MK, Khatun F, Azad AK. Anticancer activities of aceton thiosemicarbazone against ehrlich ascites carcinoma cells bearing mice. Med. J. Isl. World Aca. Sci.,2013; 21(3): 97-104.
13. Ali MM, Jesmin M, Sarker MK, Salahuddin MS, Habib MR, Khanam JA. Antineoplastic activity of N-salicylideneglycinato-di-aquanickel (II) complex against ehrlich ascites carcinoma (EAC) cells in swiss albino mice. Int J Biol Chem Sci 2008; 2: 292-298.
14. Aravindakshan KK and Nair CGR. Preparation and Characterization of Vanillin Thiosemicarbaone Complexes with Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II). Proc. Indian Acad. Sci. (Chem. Sci.), 1984; 93(2): 111-115.
15. Jesmin M. Antineoplastic and antimicrobial activities of some schiff bases and schiff base complexes. Ph.D. Thesis. Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Bangladesh 2011.
16. Clarkson BD, Buirchenal JH. Preleminary screening of antineoplastic drugs. Prog Clin Cancer 1965; 1: 625-629.
17. Hogland HC. Hematological complication of cancer chemotherapy. Semin Oncol 1982; 9: 95-102.
18. Price VE, Greenfield RE. Animea in cancer. Adv Cancer Res.1958; 5,199.
19. Burger A. Medicinal Chemistry, Vol 2 (3rd ed.), John Wiley and Sons, London, 1981; 602-653.