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IN VIVO ANTICANCER ACTIVITIES OF NI (II)-BENZOIN THIOSEMICARBAZONE COMPLEX [NI(BTSC)$_2$] AGAINST EHRLICH ASCITES CARCINOMA CELLS

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Abstract

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. In order to find out a new anticancer drug, Ni(II) complex with benzoin thiosemicarbazon was synthesized and characterized. Anticancer activities of Ni(BTSC)$_2$ has been studied against Ehrlich Ascites Carcinoma (EAC) cells in Swiss albino mice by monitoring tumor cell growth inhibition, tumor weight measurement, survival time of tumor bearing Swiss albino mice. Hematological parameters were also studied in both normal and EAC bearing treated mice. The results were compared with those obtained with a standard anticancer drug *bleomycin* and the compound was found to possess pronounced anticancer effect. Maximum cell growth inhibition was found to be 77.15% after treatment with Ni(BTSC)$_2$ at the dose of 8 mg/kg (*i.p*). About 69.56% enhancement of life span was found at 8 mg/kg (*i.p*).With the same dose Ni(BTSC)$_2$ reduced the tumor weight by 52.17% at day 20. The hematological parameters (WBC, RBC, hemoglobin content and differential counts) were found to be significantly changed as compared to those of the normal mice. These parameters restored more or less towards normal when treated with the test compound.

Key words: Antineoplastic activity, EAC cells, hematological parameters, nickel-(II) benzoin thiosemicarbazone complex, survival time

Introduction

Cancer is a diverse class of diseases which differ widely in their causes and biology. Using the new techniques of molecular biology, the causes of cancer have been searched. It seems to be the inappropriate activation of one or more proteins that regulate cells division, transforming cells to state of cancerous growth. Multidisciplinary researchers are involved to find out the causes of cancer and also developed many treatment procedures. Among them, chemotherapy is a major option. In this case schiff bases and schiff base metal complexes can create the attention of the scientist as one of the major research items to find out new, cheaper, more effective and easily available with less host toxic effects.

Schiff bases are condensation products of aldehyde and ketones with primary amines and containing imine or azomethine (-C = N-) functional group. Schiff bases are found to be a versatile pharmacophore for design and development of various bioactive led compounds. Schiff bases as well as schiff base complexes with transition metals form an important class of the most widely used organic and organometallic compounds and have a wide variety of applications in many fields including analytical, biological and inorganic chemistry. In recent times, schiff bases and schiff base metal complexes have drawn the attention of many researchers in medicinal and pharmaceutical fields due to a broad spectrum of biological activities like anticancer (Ali

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2012, 2009; Zakir 2016), antimicrobial (Jesmin 2008, Zakir 2016), anti-tubercular, anti-inflammatory and analgesic (Pandey 2011), antiviral and pesticidal (Ali 2007, Zakir 2016) activities.

In the present paper we have reported anticancer activities of $Ni(BTSC)_2$ against *Ehrlich ascites* carcinoma cells in Swiss albino mice.

Materials and Methods

Chemicals

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

Experimental animal

Swiss albino mice of 5-7 weeks old, weighing 25-30 g were selected for the work as test animals, which were collected from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR'B), Mohakhali, Dhaka.

Animal care

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

Ethical clearance

Protocol used in this study for the use of mice as animal mode for research was approved by the University Animal Ethical Committee (27/08/RUBCMB).

Synthesis of the compound

Synthesis of Ni (II)-benzoin thiosemicarbazone complex

The compound was synthesized according to the method as described in the literature (Frederick 1974, El-Shahawi 2013). For benzoin thiosemicarbazone (BTSC), benzoin and thiosemicarbazide (1:1 molar ratio) were mixed together and refluxed for a period of 3-4 hours and then distilled to half of the total volume. A saturated solution of nickel(II) acetate in ethanol was added to the condensed solution. Within a few minutes black crystals of Ni(II)-benzoin thiosemicarbazone were obtained. The crystals were then recrystallized twice, dried in an oven at 50°C and stored in a desiccator.

Characterization of the compound

The synthesized compound was characterized by taking melting point by using an electro thermal melting point apparatus, elemental analytical data were determined by using Perkin Elmer 2400 CHNS/O elemental analyzer at BCSIR Laboratory, Dhaka. The amount of metal were determined by using Atomic Absorption Spectrometer at Dhaka University and IR spectra data were obtained from Rajshahi University central laboratory as KBr disc by using a Shimadzu FTIR spectrometer.

Cell lines

EAC cells were obtained by the courtesy of Indian Institute of Chemical Biology (IICB), Kolkata, India. The cells were maintained as ascites tumor in Swiss albino mice by intraperitoneal inoculation (*i.p.*, by weekly) of 2×10^6 cells/mouse.

Toxicity study

An acute toxicity study relating to the determination of LD_{50} was performed by the conventional method (Litehifield 1949). For that purpose, the compound was dissolved in 2% dimethyl sulfoxide (DMSO) and injected intraperitoneally to six groups of mice (each containing six in number) at different doses. LD_{50} values were evaluated by recording mortality after 24 hours. The toxicity of the compound, $Ni(BTSC)_2$ has evaluated by measuring LD_{50} values and was found to be 88 mg/kg (*i.p.*).

Cell growth inhibition

In vivo tumor cell growth inhibition was carried out by the method as described earlier (Sur 1994). For this study, five groups of mice (six in each group) were used. All the mice were inoculated with 2×10^6 EAC cells intraperitoneally. Treatment was started after 24 hours of tumor inoculation and continued for 6 days. Groups 1 to 3 were treated by $Ni(BTSC)_2$ at the doses of 2 mg/kg (*i.p.*), 4 mg/kg (*i.p.*) and 8mg/kg (*i.p.*), respectively per day per mouse. Group 4 received standard drug *bleomycin* (0.3 mg/kg, *i.p.*). Treatment with only normal saline (0.98%) was considered as untreated control (Group 5). The mice of all the groups were sacrificed on the 6th day after transplantation and tumor cells were collected by repeated intraperitoneal wash with 0.98% saline. Viable tumor cells per mouse of the treated groups were compared with those of the control. The cell growth inhibition was calculated by using the following formula:

$\%$ Cell growth inhibition $= (1- T_w / C_w) \times 100$ where

T_w = Mean of number of tumor cells of the treated group of mice

C_w = Mean of number of tumor cells of the control group of mice.

Average tumor weight and survival time

The antitumor effects of $Ni(BTSC)_2$ was assessed by measuring average tumor weight, mean survival time (MST) and percentage increased of life span (% ILS) (Abbot 1976). These parameters were measured under similar experimental conditions as stated in the previous experiment (cell growth inhibition). Treatment was continued for 10 days. Tumor growths were monitored daily by measuring weight change. MST of each group (6 in each) was monitored by recording the survival time. MST and % ILS were calculated by using the following equations.

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

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

Bioassay of EAC cells

The procedure was a modification of the methods as used in literature (Fernades 1979). Two groups of mice (4 in each) were inoculated with 2×10^6 EAC cells. Group 1 was treated with $Ni(BTSC)_2$ at the doses of 2 mg/kg (*i.p.*), 4 mg/kg (*i.p.*) and 8 mg/kg (*i.p.*), respectively per day per mouse for six consecutive days. The group 2 served as control. On day 6, mice of all groups were sacrificed and tumor cells from each group

were harvested in cold saline (0.98%), pooled and centrifuged. These cells were re-inoculated (2×10^6 cells/mouse *i.p*) into two fresh groups of mice (n = 4) as before. No further treatment was done on these mice. On day 5, mice from each group were sacrificed and tumor cells per mouse were counted and compared with that of control.

Hematological studies

The hematological parameters *viz*. WBC, RBC and Hb content were determined by the standard methods using cell dilution fluids and hemocytometer (Ruisa 1988). For this purpose, blood was collected from the mouse by tail puncture. Five groups of mice (n = 4) were taken for this test. Groups 1 to 3 were treated by $Ni(BTSC)_2$ at the doses of 2 mg/kg (*i.p*.), 4 mg/kg (*i.p*.) and 8 mg/kg (*i.p*.), respectively per day per mouse. Treatment was started after 24 hours of tumor transplantation and continued for 10 consecutive days. On days 5, 10, 15 and 25 the blood parameters were assayed.

For normal mice 5 groups (n = 4) were taken for the purpose of hematological studies. The blood from the mice of group 1 was assayed on day 0 (without any treatment). Groups 2-4 were treated with $Ni(BTSC)_2$ at the doses of 2 mg/kg (*i.p*.), 4 mg/kg (*i.p*.) and 8 mg/kg (*i.p*.), respectively per day per mouse. Group 5 received standard drug *bleomycin* (0.3 mg/kg, *i.p*.).

Determination of the effect of schiff base complex $Ni(BTSC)_2$ on normal peritoneal cells

Effect of schiff base complex on normal peritoneal cells was determined by counting total peritoneal cells and number of macrophages (Hundson 1989). One group of mice (4 in each) was treated with $Ni(BTSC)_2$ at the dose of 8 mg/kg (*i.p*.) for three consecutive days. The untreated group was used as control. After 24 hours of last treatment, each animal were injected with 5 ml of normal saline (0.98%) into peritoneal cavity and then sacrificed. Intraperitoneal exuded cells and number of macrophages were counted with 1% neutral red by hemocytometer. Effect of $Ni(BTSC)_2$ complex on enhancement of normal peritoneal cells in normal mice were shown in Fig. 4.

Statistical analysis

The experimental results have been expressed as the mean ± SD. Data were calculated by ANOVA followed by Dunnett “t” test using SPSS software of 20 versions.

Results and Discussion

The synthesized compound was characterized by taking melting point and determining elemental analytical data Table 1 and 2. The structure of $Ni(BTSC)_2$ complex can be assumed to be octahedral (Fig. 1). This view is supported from the IR spectral data presented in Table 3. The ligand BTSC binds to metal ions in a mononegative tridentate fashion through C = S, C = N and OH groups (with deprotonation of OH). It is evident that the ν(C = S), ν(C = N) and ν(OH) have been shifted to lower frequency regions after bonding as compared to those for BTSC (Nakamoto et al.1971, Parashar et al. 1989). ν(C = S) band is shifted from 1263 cm^{-1} to 1130 cm^{-1}, ν(C = N) band is shifted from 1682 cm^{-1} to 1566 cm^{-1} and another coordinating site ν(OH) is also shifted from 3379 cm^{-1} to 3357 cm^{-1} after complexation (not clearly shown due to a broad spectrum)

suggesting involvement of S from C = S, N from C = N and O from OH in coordination with Ni(II) ion. Further the new bonds M-O, M-N and M-S have been detected at 617 cm^{-1} for ν (M-O), 481cm^{-1} for ν (M-N) and 422cm^{-1} for ν (M-S) respectively, which confirmed the formation of the complexes. Both the bonding and structure presented here are very much similar to those obtained earlier (El-Shahawi 2013, Ali 2011).

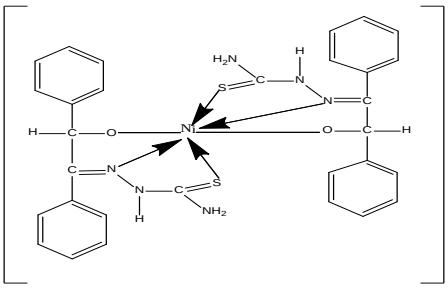


Fig. 1. Structure of $Ni(BTSC)_2$ complex.

Table 1. Yield percentage and physical characteristics of the compound.

Test compound	Yield %	Melting point °C	Physical form	Solubility
$Ni(BTSC)_2$ complex	50	Stable up to ~155°C	Black crystalline	Ethanol, Methanol, DMSO and Acetone

Table 2. Elemental analytical data of the compound.

Compound		Elemental analytical data found (calculated) in %					
		C	H	N	O	S	Metal (Ni)
$Ni(BTSC)_2$ complex	Found	52.36	4.39	12.21	4.65	9.31	16.90
	Theoretical	52.93	4.11	12.44	4.67	9.98	17.08

Table 3. IR spectral data of the compound.

Compound	*v*(OH)	*v*(C = N)	*v*(C = S)	*v*(NH-C = S)	*v*(M-O)	*v*(M-N)	*v*(M-S = C)
$Ni(BTSC)_2$ complex	3357 w	1566 s	1130 s	1029 s	617 s	524 s	422 w

[s = strong, w = weak]

In vivo tumor cell growth inhibition was observed with $Ni(BTSC)_2$ at the doses of 2 mg/kg (*i.p.*), 4 mg/kg (*i.p.*), 8 mg/kg (*i.p.*) per mouse per day. The percentage of cell growth inhibition is found to increase noticeably, with increase the doses. Maximum cell growth inhibition (77.15%) was found after treatment with $Ni(BTSC)_2$ at the dose of 8 mg/kg (*i.p*). Which is quite comparable to that of *bleomycin* at the dose of 0.3 mg/kg *(i.p.)*, when 88.20% inhibition of cell growth was observed (Table 4). The mean survival time (MST) of the untreated tumor bearing mice was 23 days. With the treatment of $Ni(BTSC)_2$, the value was found to be increased. About 69.56% enhancement of life span was found at the dose of 8 mg/kg (*i.p*), whereas the *bleomycin* showed 87.25% increase of life span at the dose of 0.3 mg/kg (*i.p.*) (Table 5).

The treatment with $Ni(BTSC)_2$ complex showed that the average tumor weight for EAC cell bearing treated mice increases at a slower rate than those of untreated EAC cell bearing mice. At day 20, $Ni(BTSC)_2$ at the dose of 8 mg/kg (*i.p.)* reduced the tumor weight by 52.17% whereas the standard drug *Bleomycin* shows 68.33% (at 0.3 mg/kg) when compared with that of control (Fig. 2).

The hematological parameters of both EAC cell bearing mice and normal mice were examined. In EAC cell bearing mice, all parameters (WBC, RBC and hemoglobin content) were found to be significantly changed as compared to those of the normal mice. These parameters restored more or less towards normal when treated with $Ni(BTSC)_2$ (Fig. 3 a-f). In case of parallel treatment of normal mice, these parameters were found to be slightly changed from normal values. After 25 days of the initial treatment, they were found to be restored to almost normal values.

The effect of $Ni(BTSC)_2$ on the loss of transplant ability of EAC cells were observed by the reduction of intraperitoneal tumor growth in mice, reinoculatated with test compound treated EAC cells (Table 6) with respect to control. Maximum reduction (52.86%) of tumor growth was observed with $Ni(BTSC)_2$ at the dose of 8 mg/kg, (*i.p.*).The compound at higher dose also enhanced both the peritoneal cells and the number of macrophages to some extent in normal mice (Table 7).

Table 4. Effect of the $Ni(BTSC)_2$ and *bleomycin* (antitumor drug) on cell growth inhibition *in vivo.*

Experiment	Dose, mg/kg (*i.p.*)	No. of EAC cells in mice on day 6 after tumor cell inoculation × 10^7	% Cell growth inhibition
Control (untreated EAC cell bearing mice)	-	2.180±0.082	-
EAC + *Bleomycin*	0.3	0.257±0.010***	88.2
	2	0.990±0.028 **	44.59
EAC + $Ni(BTSC)_2$	4	0.884±0.036 **	59.45
	8	0.498±0.051***	77.15

Mice were inoculated 2×10^6 EAC cells/mouse (*i.p.*) on days 0. Treatment was started after 24 hours of tumor cell transplantation. Number of mice in each experiment were six (n = 6); the results were shown as mean ± SEM (Standard error of mean). Treatment was continued for 6 consecutive days. Where significant values are *p <0.05, **p <0.01, and ***p <0.001 when compared with control.

Table 5. Effect of $Ni(BTSC)_2$ and *bleomycin* on survival time and increase of life span of EAC cell bearing mice.

Treatment	Dose, mg/kg (*i.p.*)	Mean survival time mean ± SEM (days)	% Increase of life span
Control (untreated EAC cell bearing mice)	-	23±0.98	-
EAC + *Bleomycin*	0.3	43±0.86***	86.95
	2	27±1.52*	17.39
EAC+ $Ni(BTSC)_2$	4	32±1.76**	39.13
	8	39±2.20***	69.56

Data are expressed as the mean of results in 6 mice ± SEM. Treatment was continued for 10 consecutive days. Where significant values are *p <0.05, ** p <0.01 and *** p <0.001 when compared with control.

Table 6. Bioassay of $Ni(BTSC)_2$ compound.

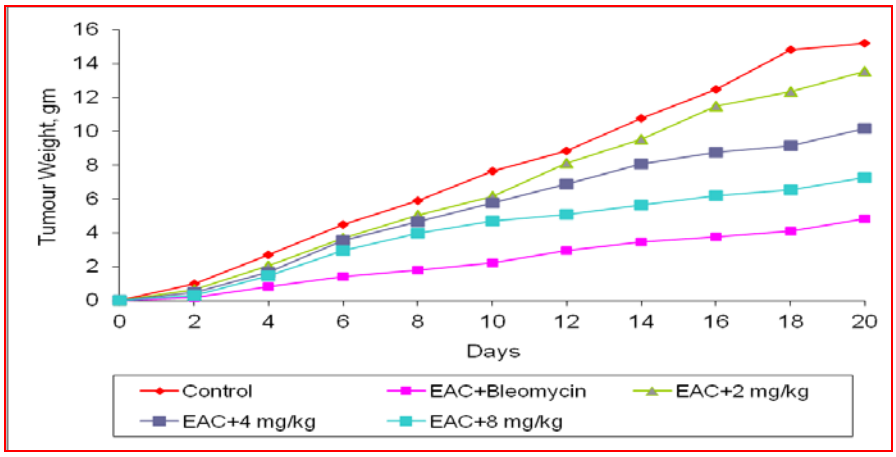
Treatment	Dose mg/kg (*i.p.*)	No. of EAC cells $\times 10^7$	Cell growth inhibition also inoculation with drug treatment EAC cell
Control (untreated EAC cell bearing mice)	-	3.14±0.04	-
	2	2.32±0.05**	26.11%
EAC+ $Ni(BTSC)_2$	4	1.90±0.09**	39.60%
	8	1.48±0.08**	52.86%

Data are expressed as the mean of results in 4 mice ± SEM Where significant values are **p <0.01 and ***p <0.001 when compared with control.

Table 7. Effect of Ni $(BTSC)_2$ on the enhancement of normal peritoneal cells in mice.

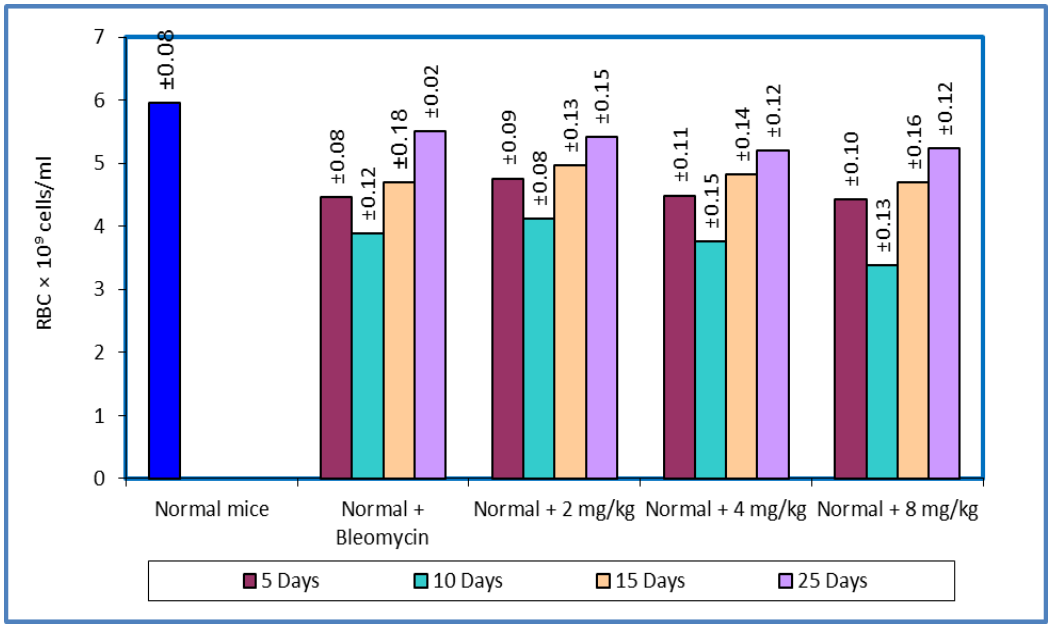
Treatment	Dose mg/kg (*i.p.*)	Macrophages (cells/mL) $\times 10^6$	Total Peritoneal cells $\times 10^6$
Control (normal)	-	1.20 ± 0.42	6.82±0.29
	2	1.90±0.28***	8.54±0.34
Normal + $Ni(BTSC)_2$	4	2.16±0.19***	9.62±0.31
	8	2.98±0.33***	10.86±0.38

Data are expressed as the mean of results in 4 mice ± SEM. Treatment was continued for 3 consecutive days. ***P <0.001 and **P <0.01 when compared with control.



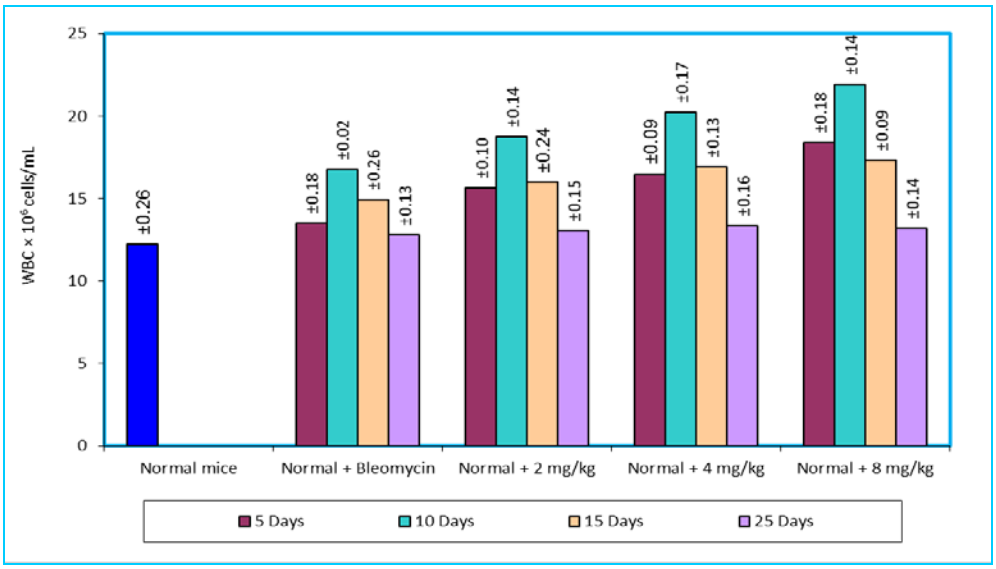
Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.

Fig. 2. Effect of $Ni(BTSC)_2$ on average tumor weight.



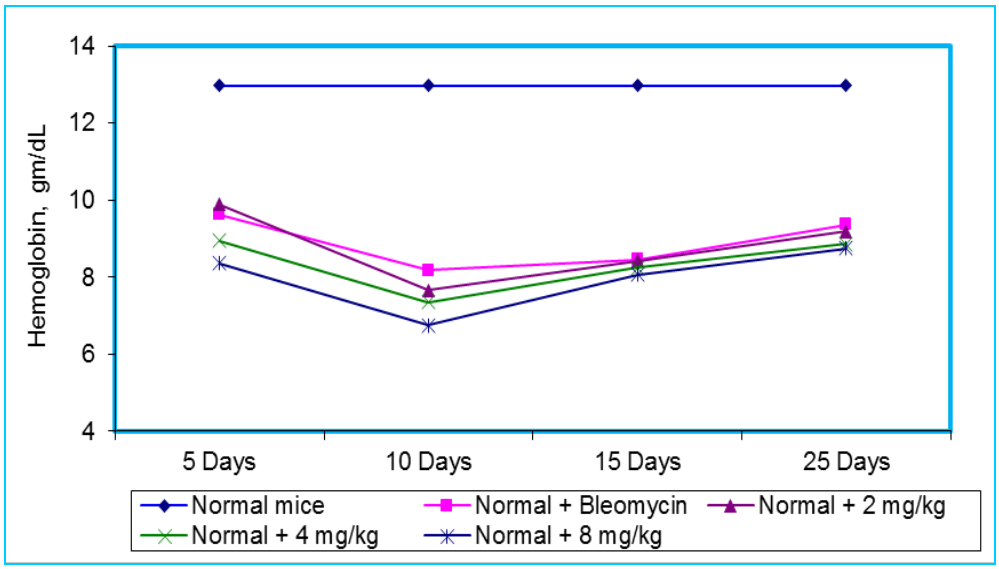
Data are expressed as the mean of results in 4 mice ± SEM. Treatment was continued for 10 consecutive days.

Fig. 3a. Effect of $Ni(BTSC)_2$ on RBC in normal mice.



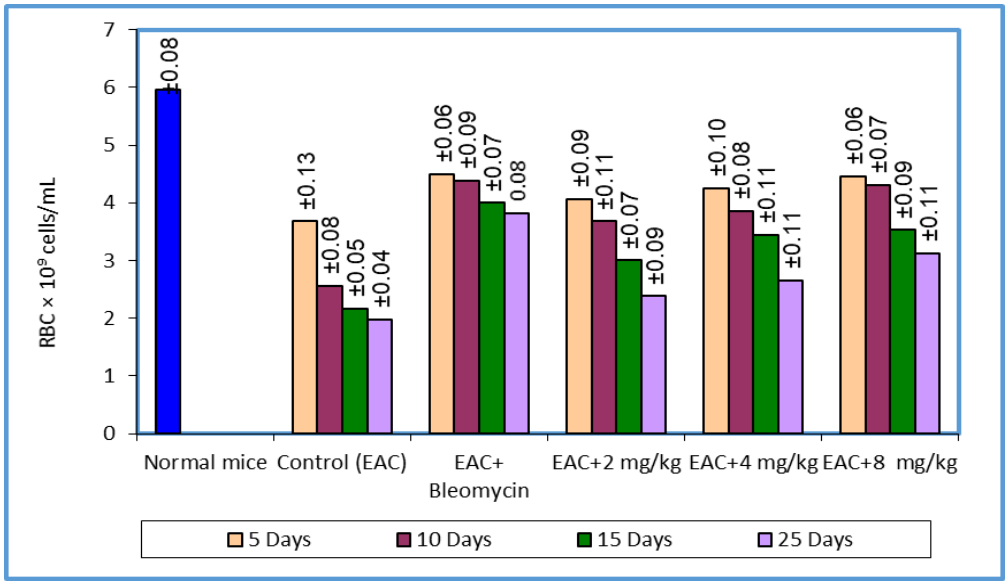
Data are expressed as the mean of results in 4 mice ± SEM. Treatment was continued for 10 consecutive days.

Fig. 3b. Effect of $Ni(BTSC)_2$ on WBC in normal mice.



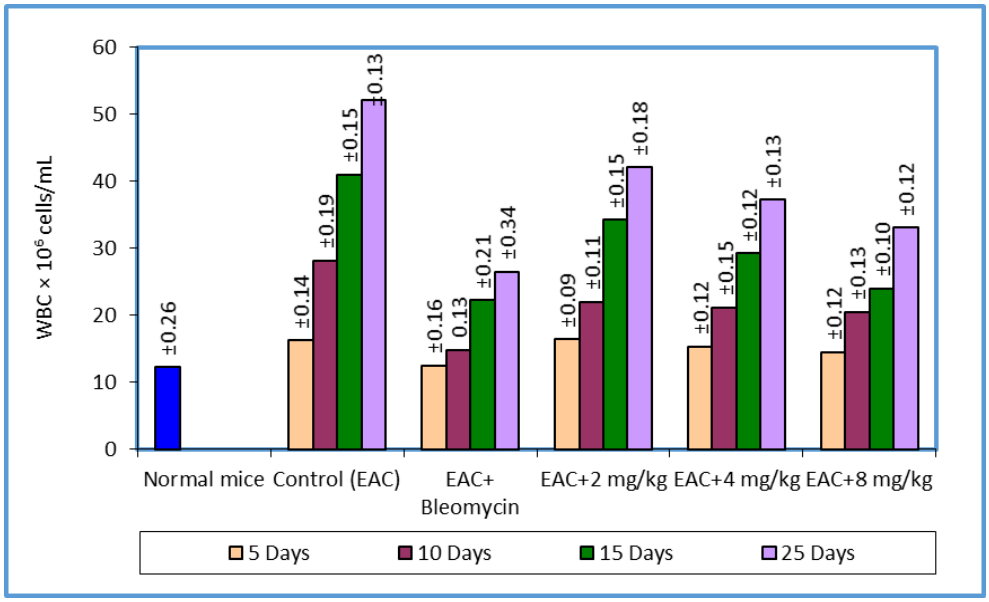
Data are expressed as the mean of results in 4 mice ± SEM. Treatment was continued for 10 consecutive days.

Fig. 3c. Effect of $Ni(BTSC)_2$ on hemoglobin content in normal mice.



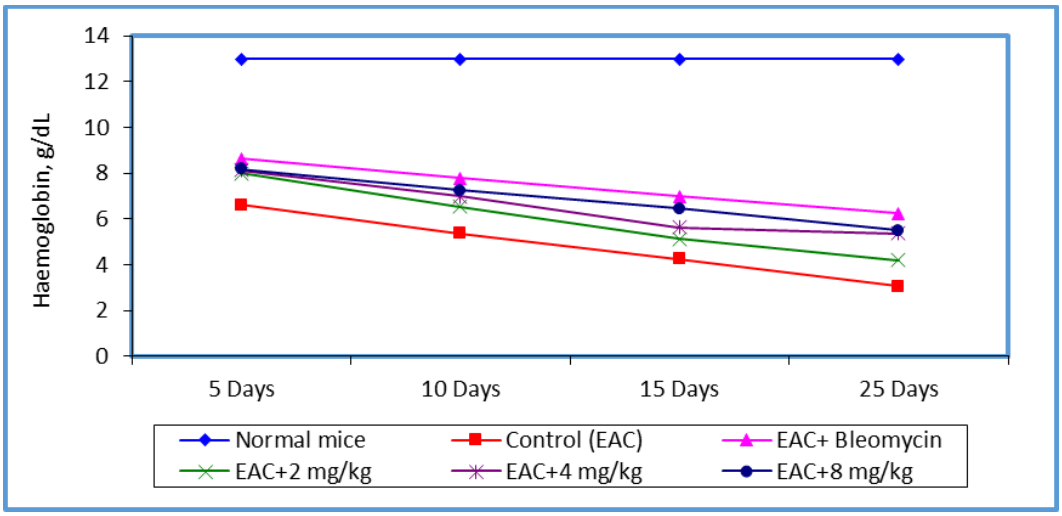
Data are expressed as the mean of results in 4 mice ± SEM. Treatment was continued for 10 consecutive days.

Fig. 3d. Effect of $Ni(BTSC)_2$ on RBC in EAC cell bearing mice.



Data are expressed as the mean of results in 4 mice ± SEM. Treatment was continued for 10 consecutive days.

Fig. 3e. Effect of $Ni(BTSC)_2$ on WBC in EAC cell bearing mice.



Data are expressed as the mean of results in 4 mice ± SEM. Treatment was continued for 10 consecutive days.

Fig. 3f. Effect of $Ni(BTSC)_2$ on hemoglobin content in EAC bearing mice.

Conclusion

The results presented in this study showed that the schiff base complex $Ni(BTSC)_2$ is capable of reducing average tumor weight and increasing life span of tumor bearing mice. It also inhibits the cell growth very successfully and restored all the hematological parameters (RBC, WBC, Hemoglobin and differential counts) more or less to normal. It also found that, Ni(BTSC)2 enhanced the number of macrophages and other peritoneal cells remarkably, so it is speculated that $Ni(BTSC)_2$ kill or destroy tumor cells by boosting cell mediated tumor immunity of the host and expected to be an effective anticancer agent with negligible toxicities. However, the information obtained from the present investigation is insufficient for $Ni(BTSC)_2$ to be used as novel anticancer drug in clinical practice. Many more investigations have to be carried out with this compound using various other cancer cell lines and higher test animals in order to confirm this as potent anticancer agents.

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