INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 4, p. 209-220, 2016

RESEARCH PAPER OPEN ACCESS

In vivo anticancer activities of Co(II)-Benzoin Thiosemicarbazone Complex [Co(BTSC)$_2$]

Hossain Mohammad Zakir*[1], Md. Khairul Islam[1], Md. Rakib Hasan[2], Shaikh M Mohsin Ali[1], Mele Jesmin[1]

[1]*Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Rajshahi, Bangladesh*

[2]*Department of Biochemistry & Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh*

Key words: Antineoplastic activity, EAC cells, Cobalt benzoin thiosemicarbazone complex, Hematological prameters, Morphological change.

http://dx.doi.org/10.12692/ijb/9.4.209-220 Article published on October 27, 2016

Abstract

Cancer, a diverse group of diseases characterized by the proliferation and spread of abnormal cells, is a major worldwide problem. In order to find out new anticancer drug Co(II) complex with benzoin thiosemicarbazone was synthesized and characterized. Ehrlich Ascites Carcinoma (EAC) cells were used to evaluate the anticancer activities of benzoin thiosemicarbazone complex with Co(II) in swiss albino mice. The anticancer activities were studied by monitoring tumor cell growth inhibition, tumor weight measurement, survival time of tumor bearing swiss albino mice. Hematological parameters of normal and EAC cells bearing treated mice were also studied. The apoptotic cell morphological changes of the treated EAC cells were confirmed by fluorescence and optical microscope. It has been found that the compound enhanced life span, reduced average tumor weight and inhibited tumor cell growth of EAC cell bearing mice remarkably. The results were compared with those obtained with a standard anticancer drug *bleomycin*. 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.

* **Corresponding Author:** Hossain Mohammad Zakir ✉ hmzakir04acct@gmail.com

Introduction

Cancer is one of the most dreaded diseases in the present time. It poses serious health problems both in developed and developing countries. It is a genetic disease promoted by some external factors like tobacco, chemicals, radiations, infectious organism and some internal factors viz mutation, hormones, immune conditions etc. It can be treated with surgery, radiation, chemotherapy, hormonal and biological therapy. The problem in Bangladesh is particularly acute because of poverty, illiteracy and others associated with poor nutrition and lack of knowledge of health matters. The prevention and control of cancer in such developing countries deserve urgent attention.

Multidisciplinary scientific investigations are making best efforts to combat this disease. Many treatment modalities have also been developed so far and of them chemotherapy is a major option. However most of these chemotherapeutic agents exhibit undesirable side effects. The drugs available so far are mostly expensive to common people.

A need is, therefore, felt to search newer remedies which are cheaper easily available with less host toxic effects. In this regard, schiff bases have already been drawn the attention of research workers (Ali MM *et al*., 2009 and 2012; Jesmin *et al*., 2010; Islam *et al*., 2012). More recently sulphur containing schiff bases like thiosemicarbazones showed better results (Liu *et al*., 1995; Ali MM *et al*., 2013, 2015; Shahriar SMS *et al*., 2014 and 2015). An antineoplastic activity of benzoin thiosemicarbazone against EAC cells has been studied in our labroratory (Ali MM *et al*., 2015). In continuation of this investigation, a complex of this schiff base with Co(II) has been synthesized and studied its anticancer activity in this present paper.

Materials and methods

Chemicals

All the chemicals used throughout the research work were purchased from British drug house (England) and used without further purification. Solvents were distilled prior use.

Experimental animal

The experimental animal was Swiss albino mice of 5-7 weeks old, weighing 25-30g. The mice were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B) Mohakhali, Dhaka.

Animal care

Mice were kept in wood box with proper bedding which was changed twice per 10 days. The room was well ventilated and the temperature was kept in 20-25°C. Standard mouse diet (recommended and prepared by International Centre for Diarrhoeal Disease Research, Bangladesh Mohakhali, Dhaka) and sufficient water were given.

Ethical clearance

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

Synthesis of the compound

Synthesis of Co(II)-benzoin thiosemicarbazone complex

The compound was synthesized according to the method as described in the literature (El-Shahawi MS *et al*., 2013).For synthesis 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 cobalt(II) acetate in ethanol was added to the condensed solution. Within a few minutes black crystals of Co(II)-benzoin thiosemicarbazone were obtained. The founding crystals were then recrystallized three to four times, then dried in an oven at 50°C and kept in a desiccator.

Structure of the compound

The structure of the synthesized compound can be assumed to be octahedral (Fig. 1). This view is supported from this IR spectral data. The ligand BTSC bind 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 $^{\upsilon}$(C=S),

υ(C=N) and υ(OH) have been shifted to lower frequency regions after bonding as compared to those for BTSC. υ(C=S) band is shifted to 1176 cm^{-1} to 1135 cm^{-1}, υ(C=N) band is shifted to 1620 cm^{-1} to 1567 cm^{-1} and another coordinating site υ(OH) is also shifted to 3420 cm^{-1} to 3339 cm^{-1} after complexation suggesting involvement of azomethine nitrogen in coordination of ligand with Co(II) ion. Further the new bond appeared for υ(Co-N), υ(Co-O) and υ(Co-S) at 427,526 and 340 cm^{-1} respectively confirmed the formation of the complex. Both the bonding and structure presented here are similar to those obtained earlier (El-Shahawi MS *et al*., 2013; Abid AMA *et al*., 2011).

Characterization of the schiff base complex

The synthesized compounds were 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 metals were determined by using Atomic Absorption Spectrometer at Department of Soil, Water and Environment, University of Dhaka.IR spectral data were obtained from central science laboratory, University of Rajshahi as KBr disc by using Shimadzu FTIR spectrometer. The data are shown in table 1, 2 and 3.

Cell lines

Ehrlich ascites carcinoma (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 JT *et al*., 1949). For the purpose the compound was dissolved in 2% dimethyl sulfoxide (DMSO) and injected intraperitoneally to six groups of mice (each containing six in number) in different doses. LD_{50} values were evaluated by recording mortality after 24 hours. The toxicity of Co(BTSC)2 has evaluated by measuring LD_{50} values and was found 75 mg/kg (i.p.).

Cell growth inhibition

In vivo tumor cell growth inhibition was carried out by the method as described (Islam K *et al*., 2012) earlier. For this study five groups of mice (six in each group) were used. All the mice were injected with EAC cells (0.1 ml of 2×10^6 cells/mouse) intraperitoneally. Treatment was started after 24 hours of tumor inoculation and continued for 6 days. Groups 1 to 3 were treated by $Co(BTSC)_2$ 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 control. The cell growth inhibition was calculated by using the following formula:

$$\% \text{ Cell growth inhibition} = \left(1 - \frac{I_w}{C_w}\right) \times 100$$ 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 $Co(BTSC)_2$ was examined by measuring mean survival time (MST), percentage increase of life span (% ILS) and average tumor weight. These parameters were measured under similar experimental conditions as stated in the previous experiment (cell growth inhibition). Treatment was continued for 10 days, Tumor growth was 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.

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

Percent increase of life span, % 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 (Abbot BJ *et al*., 1976). Two groups of mice (4 in each) were inoculated with 2×10^6 EAC cells. Group 1 was treated with $Co(BTSC)_2$ at the dose of 8 mg/kg (*i.p*) for five consecutive days. The group 2 served as control. On day 6 experimental mice of all groups were sacrificed and collected tumor cells which were then harvested in cold saline (0.98%), pooled and centrifuged. These cells were re-inoculated (2×10^6 cells/mouse *i.p*) in to 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. White blood cell, Red blood cell and Heamoglobulin content were determined by the standard methods (Ruisa V *et al*., 1988**)** using cell dilution fluids and hemocytometer. 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 $Co(BTSC)_2$ 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 started after 24 hours of tumor transplantation and was 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. The blood from the mice of group 1 was assayed on day 0 (without any treatment). Groups 2-4 were treated with $Co(BTSC)_2$ at the same doses for the same days and same way. Group 5 received standard drug *bleomycin* (0.3 mg/kg, *i.p*.).

Determination of the effect of schiff base complex on normal peritoneal cells

Effect of schiff base complex on normal peritoneal cells was determined (Hundson L *et al*., 1989) by counting total peritoneal cells and number of macrophages.

A group of mice (4 in each) was treated with $Co(BTSC)_2$ for three consecutive days at the dose of 8 mg/kg (*i.p*.).

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. Number of macrophages and intraperitoneal exuded cells were counted with 1% neutral red by hemocytometer. Effect of $Co(BTSC)_2$ complex on enhancement of normal peritoneal cells in normal mice were shown in figure 4.

Cell morphologic change and nuclear damage

Morphological observation of cells in absence and presence of experimental compound was studied using a fluorescence microscope (Olympus iX71, Seoul, Korea). Tumour cells were collected from treated Swiss albino mice by $Co(BTSC)_2$ (8 mg/kg/day) and untreated EAC cell bearing Swiss albino mice and washed twice with phosphate buffer saline (PBS). Then cells were stained with 4', 6-diamidino-2-phenylindole (DAPI) and washed twice again with PBS. Effect of $Co(BTSC)_2$ on morphological changes of EAC cells were shown in figure 5.

Statistical analysis

The experimental results have been expressed as the mean S.E.M. Data have been calculated by one was ANOVA followed by Dunnett “t” test using SPSS software of 20 version.

Results

In vivo tumor cell growth inhibition was observed with $Co(BTSC)_2$ at the doses of (2 mg/kg i.p., 4 mg/kg i.p., 8 mg/kg i.p.) per mouse per day. Maximum cell growth inhibition (85.69%) was found after treatment with $Co(BTSC)_2$ at the dose of 8 mg/kg (*i.p*). On the other hand *bleomycin* at the dose of 0.3 mg/kg (i.p) inhibited the cell growth by 88.2% (Table 4).

Table 1. Yield percentage and physical characteristics of the schiff base complex.

Test compound	Yield %	Melting point °C	Physical form	Solubility
$Co(BTSC)_2$ complex	45	Stable up to ~155°c	Gray crystalline	Ethanol, Methanol, DMSO and Acetone.

Table 2. Elemental analytical data of the schiff base complex.

Compound		Elemental analytical data found (calculated) in %					
		C	H	N	O	S	Metal (Co)
Co(BTSC)2 complex	Found	39.17	4.01	5.26	20.96	12.55	17.02
	Theoretical	39.75	4.05	5.15	21.60	13.25	17.13

The mean survival time (MST) of the untreated tumor bearing mice was 23 days. With the treatment of test compound, the value was increased. About 82.61% enhancement of life span was found at 8 mg/kg (*i.p*) with $Co(BTSC)_2$. (Table 5).

The treatment with schiff base complex also reduced the rate of tumor growth. At day 20, $Co(BTSC)_2$ at the dose of 8 mg/kg i.p reduced the tumor weight by 62.55% as compared to that of control mice. *Bleomycin* at dose 0.3 mg/kg i.p. reduced the same by 73.83% (Fig. 2.).

Table 3. Infrared spectral data of the schiff base complex.

Compound	$\upsilon(NH_2)$	υ(N-H)	υ(C=N)	υ(C=S)	υ(C=O)	υ(NH-C=S)	υ(M-O)	υ(M-N)	υ(M-S=C)
$Co(BTSC)_2$ complex	3339 w	-----	1567 s	1135 s	------	682-1024 br	526 w	427 s	340 s

[s= strong, w= weak, m= medium,]

Table 4. Effect of the $Co(BTSC)_2$ 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
EAC + $Co(BTSC)_2$	2	0.950±0.019***	56.42
	4	0.554±0.016***	74.58
	8	0.312±0.007***	85.69

Approx. 2×10^6 EAC cells/mouse (*i.p.*) was inoculated 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 ± S.E.M (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.

The hematological parameters of both normal mice and EAC cell bearing mice were examined. In EAC cell bearing mice all parameters (White blood cell, Red blood cell, heamoglobin 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 (Fig. 3A. to 3F.).

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 $Co(BTSC)_2$ 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 (59.87%) of tumor growth was observed with $Co(BTSC)_2$ (8 mg/kg, i.p.).

The compound at high dose also enhanced both the peritoneal cells and the number of macrophages to some extent in normal mice (Table 7).

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

Treatment	Dose, mg/kg (*i.p.*)	Mean survival time mean ± S.E.M. (days)	% Increase of life span
Control (untreated EAC cell bearing mice)	-	23±0.98	-
EAC + *Bleomycin*	0.3	43±0.86***	86.95
EAC+ $Co(BTSC)_2$	2	30±0.75*	30.43
	4	38±0.87**	65.22
	8	42±1.12**	82.61

Data are expressed as the mean of results in 6 mice ± S.E.M. 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 $Co(BTSC)_2$ complex.

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	-
EAC+ $Co(BTSC)_2$	2	2.08±0.006***	33.76%
	4	1.82±0.004***	42.15%
	8	1.26±0.007***	59.87%

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

Discussion

The results presented in the above section showed that the schiff base complex $Co(BTSC)_2$ is capable of increasing life span of tumor bearing mice and also reduced average tumour weight. In all cases, these abilities increase with increased doses of the compound.

With 8 mg/kg (*i.p.*) $Co(BTSC)_2$ showed maximum results which are quite comparable to those of *bleomycin* at dose 0.3 mg/kg (i.p.). Analogous results have been obtained for EAC cell growth inhibition of the schiff base complex. With the increase of doses the percentage of cell growth inhibition is found to increase noticeably.

Table 7. Effect of $Co(BTSC)_2$ complex 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
Normal + $Co(BTSC)_2$	2	2.35±0.29***	10.19±0.28
	4	3.65±0.30***	11.69±0.26
	8	4.25±0.30***	12.32±0.26

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

Table 8. Comparative results on anticancer activity of different compounds obtained by different coworkers.

Name of the compounds	Dose, mg/kg (*i.p.*)	% of Cell growth inhibition	Mean survival time mean ± S.E.M. (days)	% increase of life span
EAC + *Bleomycin*	0.3	88.2	43±0.86***	86.95
EAC + BTSC	8	73.53	32±1.18**	47.30
EAC+ $Co(BTSC)_2$	8	85.69	42±1.12**	82.61

Where significant values are ** p < 0.01 and *** p < 0.001 when compared with control.

In justifying the potency of the compound in cancer chemotherapy all the above results are considered as very important and promising aspects. (Clarkson BD *et al*., 1965). In cancer chemotherapy the major problems which are usually encountered were myelosuppression and anemia (Hogland HC *et al*., 1982; Price VE *et al*., 1958**)** due to the decrease of both red blood cell and hemoglobin content.

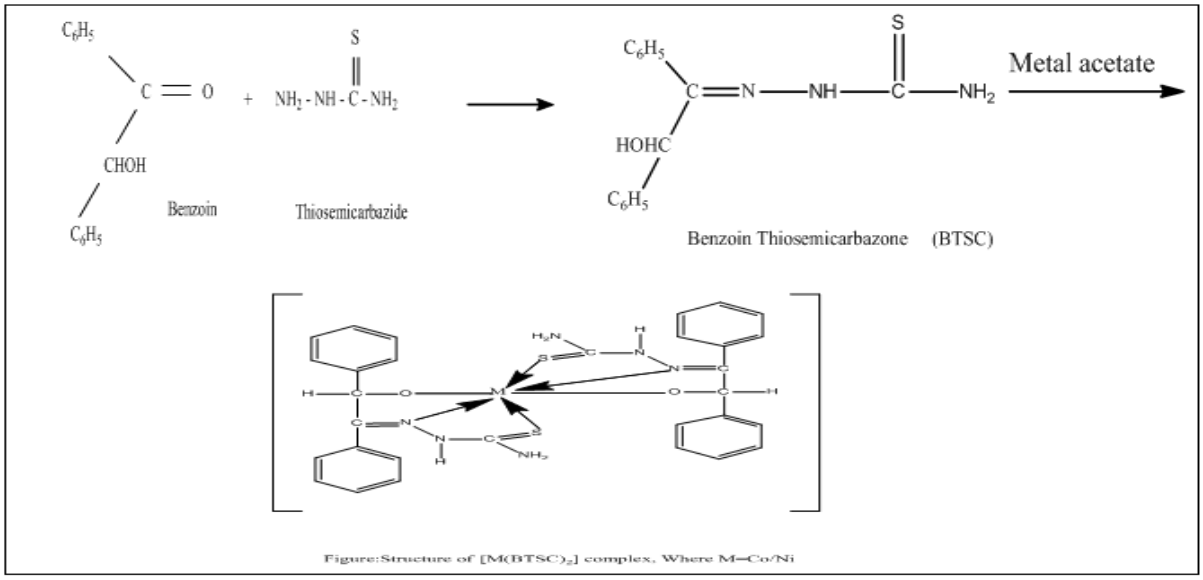

Fig. 1. Synthesis of metal (II)-benzoin thiosemicarbazone complexes, M(II) stands for Co(II)/Ni(II).

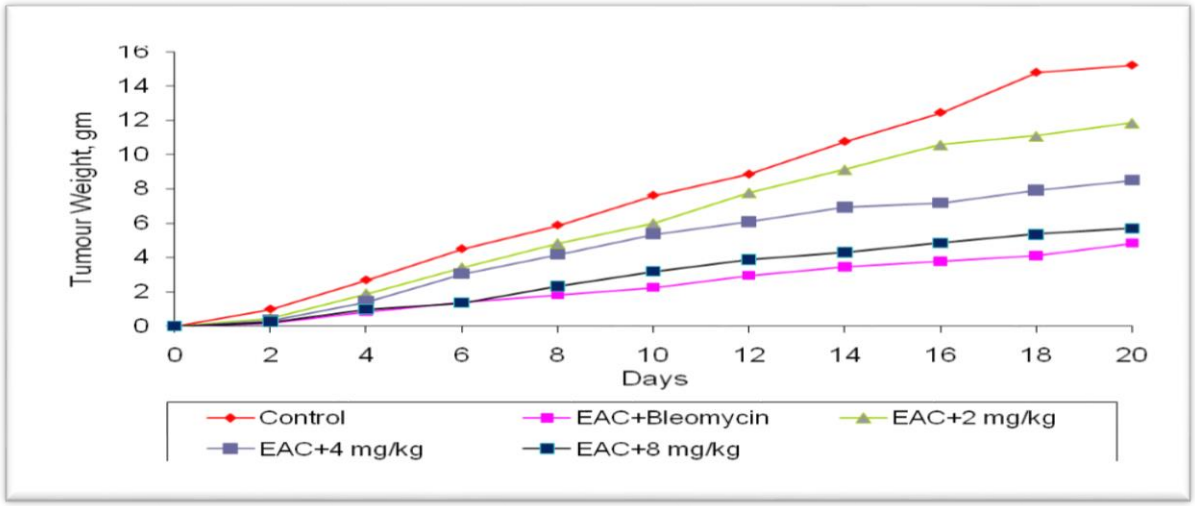

Fig. 2. Effect of $Co(BTSC)_2$ on average tumor weight. Data are expressed as the mean of results in 6 mice. Treatment was continued for 10 consecutive days.

This is probably owing to the deficiency of iron on hemolytic or myelopathic condition (Fenninger LD *et al*., 1954). After treatment with the synthesized compound under investigation, all the depleted hematological parameters can be reversed towards normal. The host toxic effects of the schiff base complex are not very high. This indicates that $Co(BTSC)_2$ possess protective actions on the hemopoietic system.

The rectifying ability for the hematological parameters of EAC bearing mice has definitely been demonstrated by the increase of life span and such other parameter studied for evaluating the potency of the compound as antineoplastic agent.

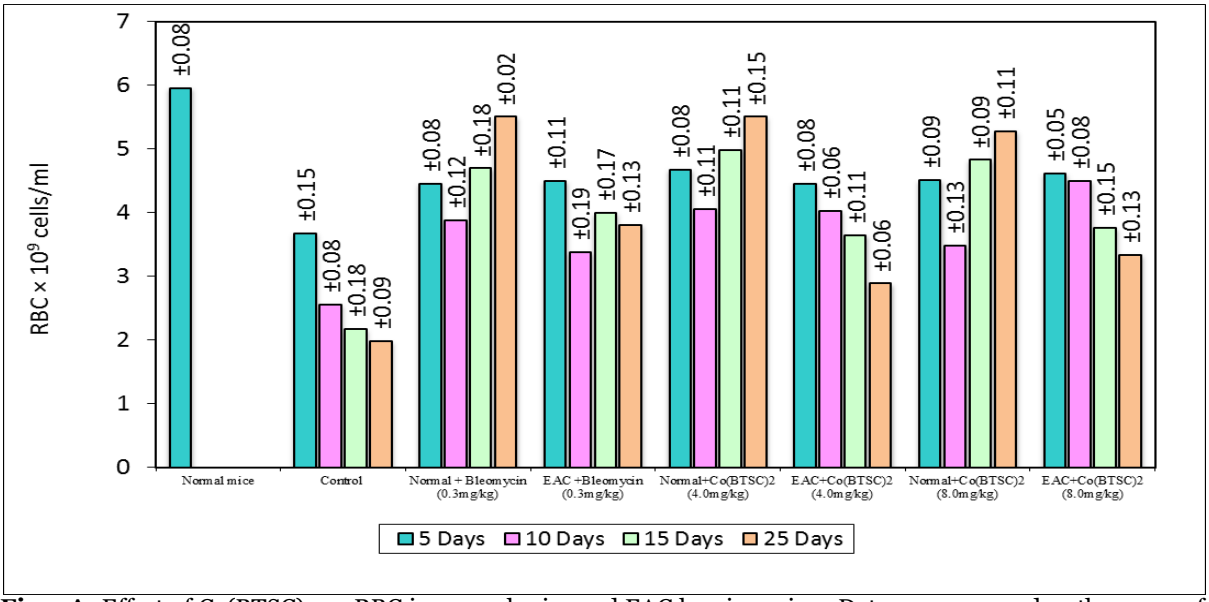

Fig. 3A. Effect of $Co(BTSC)_2$ on RBC in normal mice and EAC bearing mice. Data are expressed as the mean of results in 4 mice ± S.E.M. Treatment was continued for 10 consecutive days.

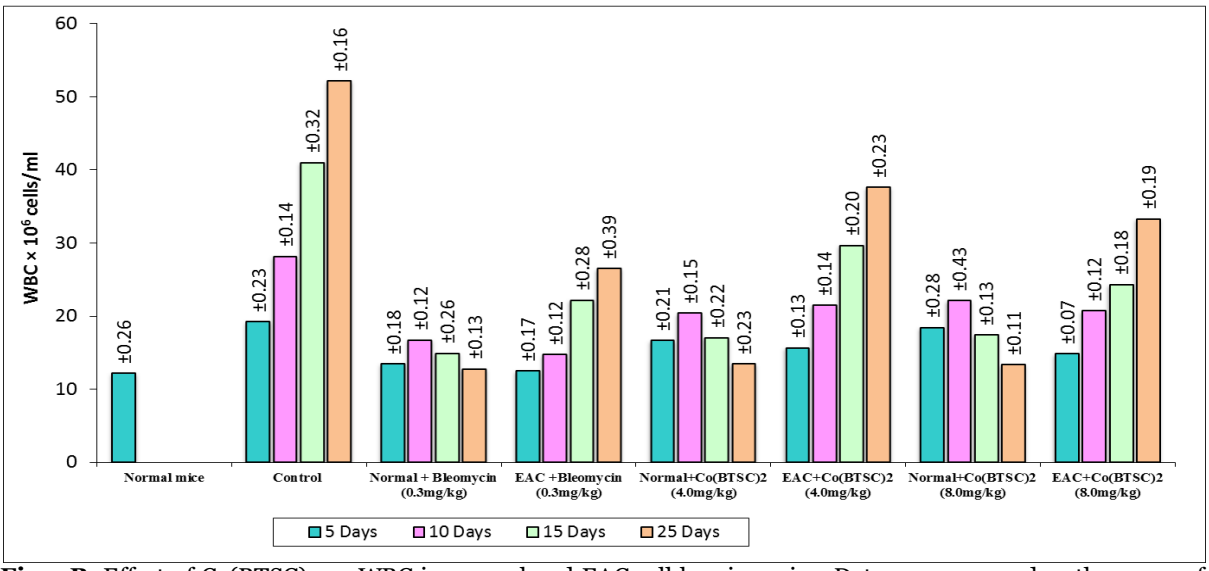

Fig. 3B. Effect of $Co(BTSC)_2$ on WBC in normal and EAC cell bearing mice. Data are expressed as the mean of results in 4 mice ± S.E.M. Treatment was continued for 10 consecutive days.

Effects of $Co(BTSC)_2$ on viability of EAC cells are found to be reduced significantly. In addition the treatment in normal mice increases the macrophages and peritoneal cells which have also been considered as a very vital event in acquiring self-destroying activities of the living being towards cancer cells (Fernades DJ *et al*., 1979).

Enhancement of macrophases might produce some cytokines such as tumor necrosis factors (TNF) and interleukins inside the peritoneal cavity, which in turn may be responsible for killing tumor cells (Lee NN *et al*., 1982).

The schiff base complex enhances the number of peritoneal macrophages significantly. High LD_{50} value of this compound represents the low toxicity of the compound to the host.

Finally, it has been found that the results presented here are somewhat better than those found eailer (Ali MM *et al*., 2015).

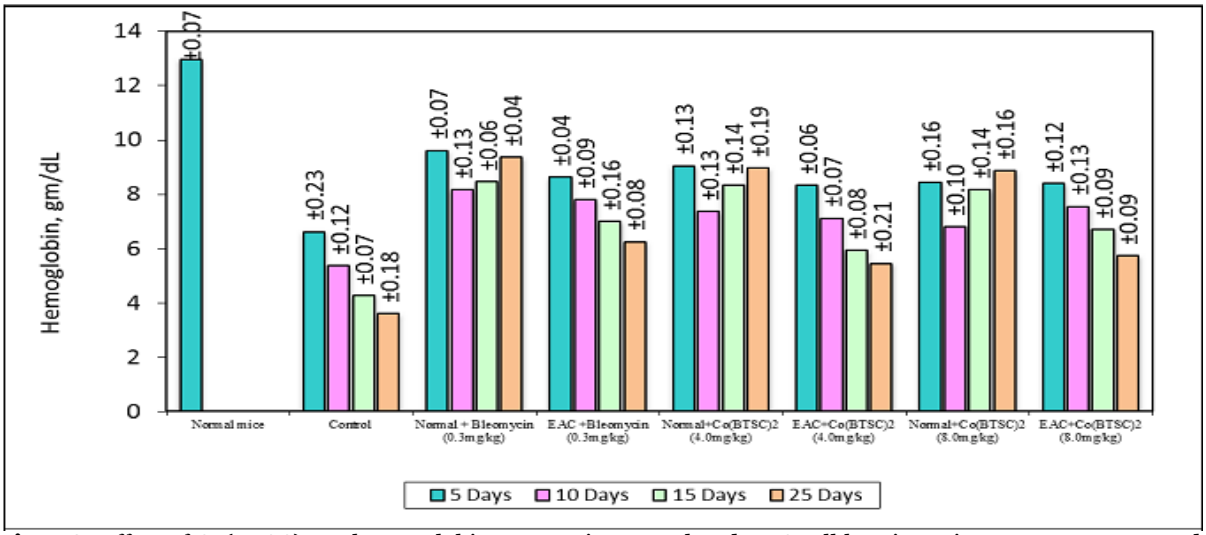

Fig. 3C. Effect of $Co(BTSC)_2$ on heamoglobin content in normal and EAC cell bearing mice. Data are expressed as the mean of results in 4 mice ± S.E.M. Treatment was continued for 10 consecutive days.

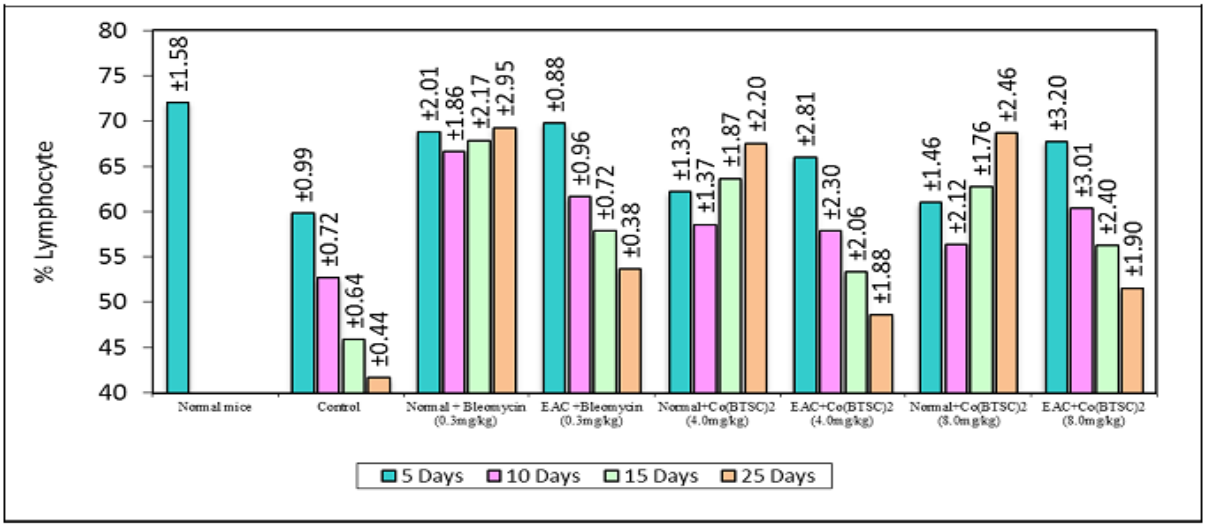

Fig. 3D. Effect of $Co(BTSC)_2$ on lymphocyte content in normal and EAC cell bearing mice. Data are expressed as the mean of results in 4 mice ± S.E.M. Treatment was continued for 10 consecutive.

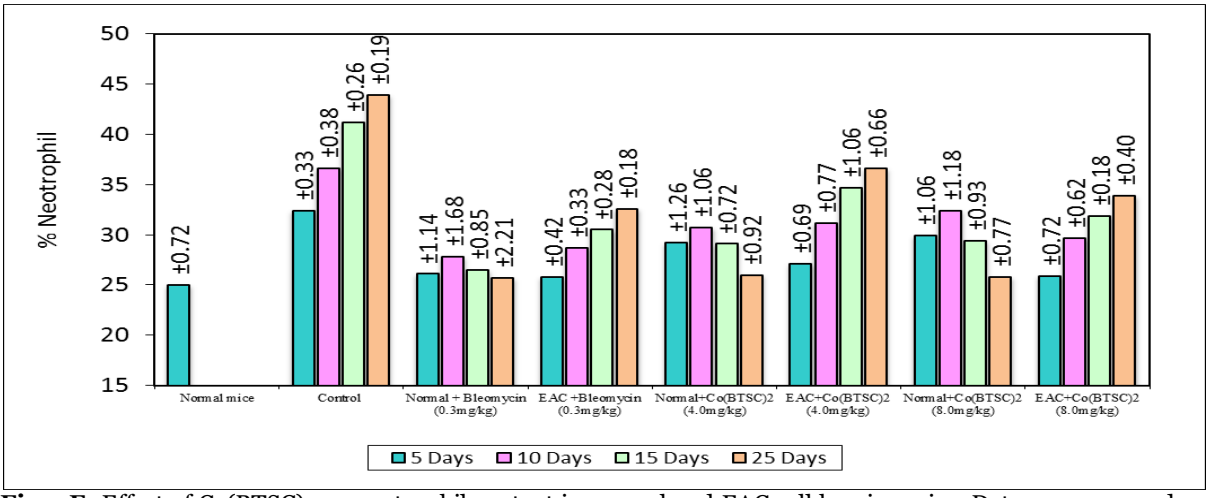

Fig. 3E. Effect of $Co(BTSC)_2$ on neutrophil content in normal and EAC cell bearing mice. Data are expressed as the mean of results in 4 mice ± S.E.M. Treatment was continued for 10 consecutive

A comparative data is given below (Table 8). Better results are expected probably due to the reduction of the polarity of the cobalt ion by partial sharing of the positive charge with the ligands (BTSC) donar atoms so that there is electron delocalization within the metal complex. This might increase the lipophilic character of the complex, enabling it to permeate the lipid layer of the organism killing them more effectively.

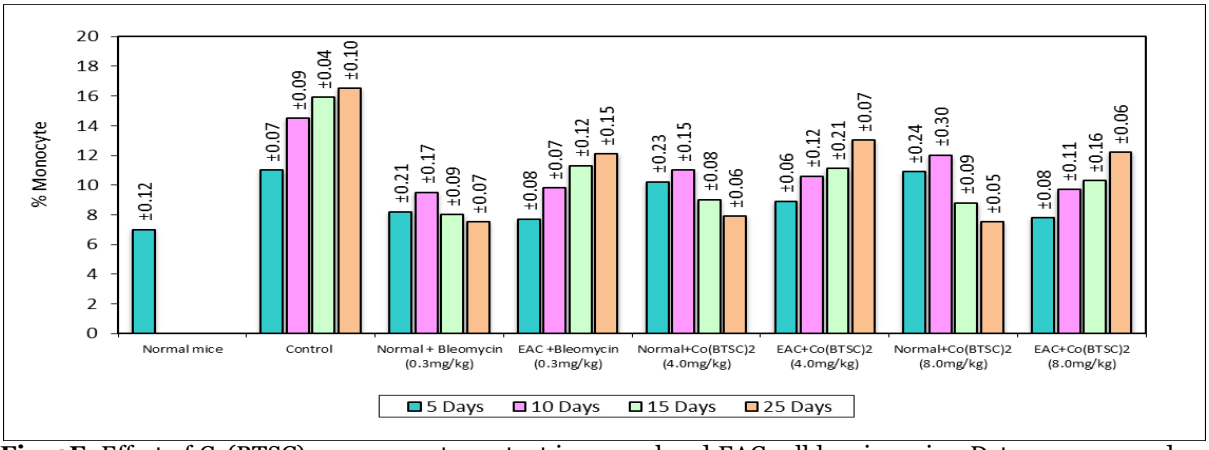

Fig. 3F. Effect of $Co(BTSC)_2$ on monocyte content in normal and EAC cell bearing mice. Data are expressed as the mean of results in 4 mice ± S.E.M. Treatment was continued for 10 consecutive.

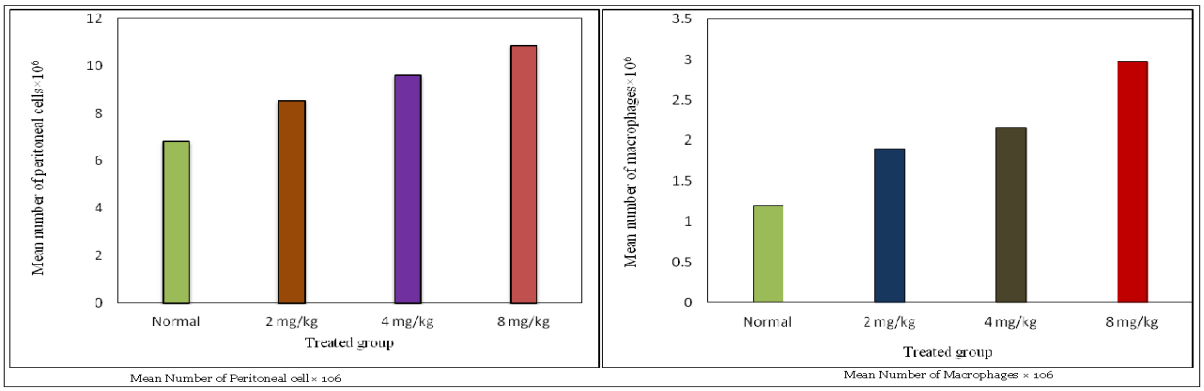

Fig. 4. The effect of $Co(BTSC)_2$ complex on enhancement of normal peritoneal cells in normal mice

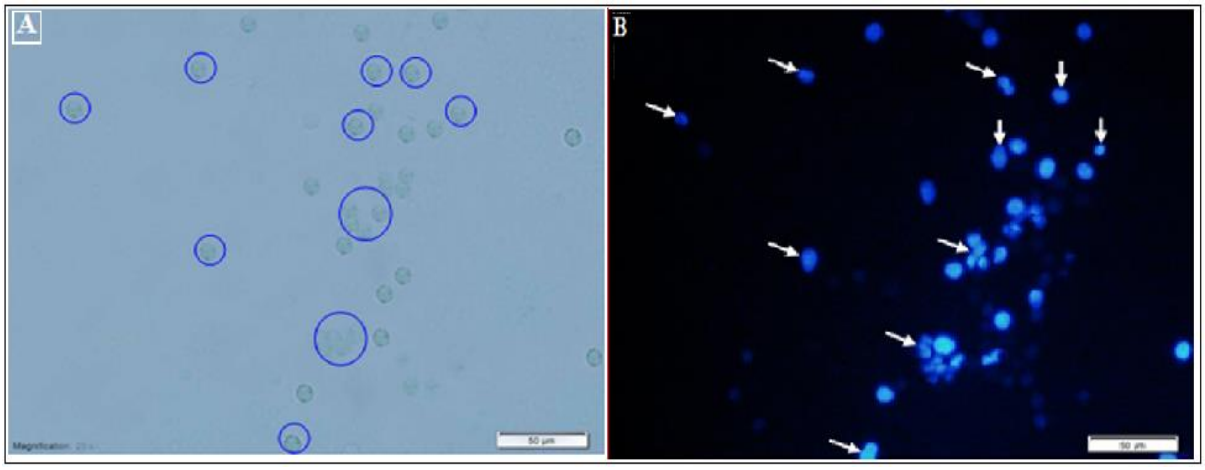

Fig. 5. Effect of $Co(BTSC)_2$ [8 mg/kg/day (i.p.)] on morphological changes of EAC cell. Cells were collected from untreated EAC cell bearing mice A (optical) and $Co(BTSC)_2$ treated EAC cell bearing mice B (fluorescence). Arrows indicating apoptotic features (condensed chromatin and nuclear fragmentation).

Conclusion

The discovery and developments of new potent and selective anticancer drugs are of high importance in modern cancer researches. From the above results and discussion it is expected that $Co(BTSC)_2$ will be an effective anticancer agent with low toxicities. However, the data obtained from the present study is insufficient for $Co(BTSC)_2$ to be used as novel chemotherapeutic agent 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.

Abbreviations

Kilogram: *kg* Milligram: *mg*
Micrometer: *mm* Molar: *mol/L*
Milliliter: *ml* Benzoin thiosemicarbazone: *BTSC*

References

Ali MM, Jesmin M, Mondal S, Rashed A and Islam K. 2015. *In vivo* Antineoplastic Activities of Benzoin Thiosemicarbazone and Para-Anisaldehyde Semicarbazone against Ehrlich Ascites Carcinoma Cells. Medical Journal of Islamic World Academy of Sciences **23(4),** 131-139.

Ali MM, Jesmin M, Islam MK, Fatema K, Azad KA. 2013. Antineoplastic activities of acetone thiosemicarbazone against Ehrlich ascites carcinoma cells bearing mice. Medical Journal of Islamic World Academy of Sciences **21(3),** 97-104.

Ali MM, Azad MAK, Jesmin M, Ahsan S, Rahman MM, Khanam JA, Islam MN, Shahriar SMS. 2012. *In vivo* anticancer activity of vanillin semicarbazone. Asian Pacific Journal of Tropical Biomedicine **2(6),** 438-442. http://dox.doi.org/10.1016/S2221-1691(12)60072-0

Ali MM, Jesmin M, Islam MN, Shahrair SMS, Habib MR, Islam MF. 2009. Anticancer activities of some transition metal complexes of a schiff base derived from salicylaldehyde and glycine. ACGC Chemical Research Communications **23,** 13-22.

Abbot BJ. 1976. Bioassay of plant extract for anticancer activity. Cancer Treatment Reports **60,** 1007-1017.

Abid AMA, Methak SM, Muna AH. 2011. Preparation and Characterization of some transition metal complexes with new Schiff base ligand derived from benzophenone. Journal of Kufa for Chemical science. NO. (2).

Clarkson BD, Buirchenal JH. 1965. Preliminary screening of antineoplastic drugs. Progress in Clinical Cancer **1,** 625-629.

El-Shahawi MS, Al-Jahdali MS, Bashammakh AS, Al-Sibaai AA, Nassef HM. 2013. Spectroscopic and electrochemical characterization of some Schiff base metal complexes containing benzoin moiety. Acta Part A: Molecular and Bimolecular Spectroscopy **113,** 459–465. http://dox.doi.org/10.1016/j.saa.2013.04.090

Fernades DJ, Klubes P. 1979. A biochemical and pharmacological study of therapeutic synergism with 5-fluonounacil plus cyclophosphamide in murine L_{1210} Leukemia. Cancer Research **39,** 1396-1404.

Fenninger LD, Mider GB. 1954. Energy and nitrogen metabolism in cancer. Advance Cancer Research **2,** 2229-2253. http://dox.doi.org/10.1016/S0065-230X (08)60496-0

Hogland HC. 1982. Hematological complication of cancer chemotherapy. Semin Oncology **9,** 95-102.

Hundson L, Hay FC. 1989. Isolation of normal peritoneal macrophages. In practical Immunology, 3rd ed. Black Well Science publisher. Oxford, London, p.26.

Islam K, Ali MM, Jesmin M, Khanam JA. 2012. *In vivo* anticancer activities of benzophenone semicarbazone against Ehrlich ascites carcinoma (EAC) cells in swiss albino mice. Cancer Biology and Medicine **9,** 242-247. http://dox.doi.org/10.7497/j.issn.20953941.2012.04.004

Jesmin M, Ali MM, Khanam JA. 2010. Antitumor activities of some schiff bases derived from benzoin, salicylaldehyde, amino phenol and 2, 4, dinitrophenyl hydrazine. Thai Journal of Pharmaceutical Sciences **34,** 20-31.

Liu MC, Lin TS, Penketh P, Sartorelli AC. 1995. Synthesis and antitumor activity of 4- and 5-substituted derivatives of isoquiniline-1-carboxaldehyde thiosemicarbazone. Journal of Medicinal Chemistry **38(21),** 4234-43.

Litehifield JT, Wilcoxon FA. 1949. Simplified method of evaluation dose effects experiment. Journal of Pharmacology and Experimental Therapeutics **96(2),** 99-113.

Lee NN, Cadman EC, Michael IN, Michael C, Joseph R, Bertino Farber LR, Prosnitz LR. 1982. Randomized study comparing doxorubicin, cyclophosphamide, vincristine, methotrexate with leucovorin rescue and cytarabine (ACOMLA) with cyclophosphamide, doxorubicin, vincristine, prednisone and bleomycin (CHOP-B) in the treatment of diffuse histiocytic lymphoma. Cancer Treatment Reports **66(6),** 1279-1284.

\

Price VE, Greenfield RE. 1958. Anemia in cancer. Advance Cancer Research **5,** 199-200.

Ruisa V, Sood SK. 1988. Routine hematological tests In Medical Laboratory Technology. Mukherjee K J (ed.) Tata McGraw Hill Comp. Ltd. New Delhi, 218.

Shahriar SMS, Ali MM, Jesmin M. 2015. In vivo anticancer activities of vanillin thiosemicarbazone Complexes with Co(II) and Ni(II).Medical Journal of Islamic World Academy of Sciences **23(3),** 90-95.

Shahriar SMS, Ali MM, Jesmin M, Islam MK, Mondal S. 2014. In vivo anticancer activity of vanillin, benzophenone and acetophenone thiosemicarbazones on Swiss albino mice. Journal of Coastal Life Medicine **2(10),** 811-816. http://dx.doi.org/10.12980/JCLM.2.201414B35