

Research article

Evaluation of antitumor and anticancer activity of 4-amino benzoic benzoyl benzimidazole in ehrlich's ascites carcinoma induced male swiss mice

Amit Samanta, Himangshu Sekhar Maji, Abhijit De*, Sudip Kumar Kar

Department of Pharmaceutical Technology, Bengal School of Technology, Sugandha, West-bengal, India.

Key words: 4-amino benzoic benzoyl benzimidazole, Cancer, Carcinoma, Ehrlich ascites carcinoma.

***Corresponding Author: Abhijit De,**
Department of Pharmaceutical Technology,
Bengal School of Technology, Sugandha,
West-bengal, India.

Abstract

Experimental tumors by Ehrlich ascites carcinoma have gained attention for the induction of carcinoma in swiss albino mice. Cancer is one of the most emerging life threatening factors in developing countries. Presently various benzimidazole derivatives have drawn the attention as most active heterocyclic compounds with various pharmacological activities. In the present study, the benzimidazole derivative, 4-amino benzoic benzoyl benzimidazole (4ABBB) was synthesized and acute toxicity (LD_{50} = 250mg/kg body weight which was administered orally) of 4ABBB on swiss albino mice were determined. The protocol started with the tumor induction in animals by inoculating 2×10^6 tumor cells and then they are divided into five groups of ten animals each. After 24 h of transplanting, the drug was administered intraperitoneally at a dose of 20mg/kg and 10mg/kg body weight and 20 mg/kg bodyweight 5-Fluorouracil as reference drug. At the tenth day, half of the 18 h fasted animals were sacrificed and i.p fluid was collected to determine tumor volume, weight, viable (cancer) and nonviable (dead) cell count. Blood was collected to determine hemoglobin concentration, RBC count, WBC count and percentage of packed cell volume. Half of the remaining animals in each group were kept for assessing percentage increase in life span (ILS) and mean survival time. 4ABBB showed a significant improvement in a dose dependent manner especially, reduction in viable cell count ($P < 0.001$), RBC count and hemoglobin concentration and improvement in percentage of life span which is comparable with 5-Fluorouracil.

Introduction

Benzimidazole derivatives occupy a great center of importance with diverse biological activity and clinical relevance. Previously various derivatives of benzimidazole had been synthesized to figure out the potential pharmacophore against various disorders. They are usually prepared by reacting aromatic o-diamines and carboxylic acid or aldehydes under various synthetic conditions [1]. Novel derivatives are designed by altering either in their side chains or functional groups. Currently, a variety of potent benzimidazole derivatives have found pharmacological applications, for eg, 2-substituted benzimidazole derivative as antimicrobial [2], 2-(6-fluorochroman-2-yl)-1-alkyl/acyl/aryloxy-1H-benzimidazoles as antibacterial, 20-aryl substituted-1H, 10H-[2, 50]-bisbenzimidazolyl-5-carboxamidines and 2-substituted benzimidazole derivatives as antifungals [3], 2-arylbenzimidazoles, 4-[1-(substituted aryl/alkyl carbonyl)-benzimidazol-2-yl]-benzene sulphonic acids and (L)-2-(pyridin-2-yl)-N-(2-(4-nitrophenyl) pentan-

3-yl)-1H-benzimidazole-4-carboxamide as antivirals [4], N-substituted-2-(5-nitrofuranyl or 5-nitrothiophen-2-yl)-3H-benzo[d]imidazole-5-carbohydrazide derivatives as antimalarial [4], omeprazole and lansoprazole (proton pump inhibitors) as antiulcers [5], substituted 2-trifluorobenzimidazoles and 2-(trifluoromethyl)-1H-benzimidazole derivatives as antiprotozoals [6] [7], 2-(Trifluoromethyl)-1H-benzimidazole as antiparasitic [8], 2-methylamino-benzimidazole derivatives as anti-inflammatory [8], N-alkoxy-2-alkyl-benzimidazoles as HIV inhibitors [9] and 5, 6 dichloride benzimidazole derivatives as androgen receptor antagonist [10].

Cancer is uncontrolled and abnormal proliferation of cells, which become a serious life threatening issue regarding human health. Various class of anticancer drugs or chemotherapeutic agents are available widely which either undergoes alkylation of DNA or microtubules arrest or altering the cells at various mitotic phases. Those agents persist with high toxicity to host body due to their direct intercalation with the host DNA [11]. Chemically

synthesized benzimidazole derivatives earn a great value as antitumor, antiproliferative and anticancer agents. Previously researches on nitrobenzimidazoles as antitumor [11], 2-(*o*-bromobenzylamino)-1-cinnamoylbenzimidazole as antiproliferative [12] and 1, 3-diarylpiazinobenzimidazole derivatives as anticancer agents [13] were carried out. They were targeted against various cell lines including MCF-7, HCT-116, VERO, HeLa, HEP-2 (Human Larynx Cancer Cell line) and Ovarian cell line with quantification of IC50 values [14] [15].

Ehrlich ascites carcinoma (EAC) is used as a potent experimental tumor inducer by subcutaneous transplantation of tumor tissues from mouse to mouse. These cells rapidly grow as translucent suspension within the peritoneal cavity of mice. Moreover no cells get adhered to the synthetic surface *in vitro* [15] [16] [17]. The ascites fluid is accumulated in an amount of 5 or 12 cc in 4 or 6 days. After inoculation into the peritoneal cavity of mice, these cells grow majorly in four phases: firstly number of cells will increase exponentially termed as proliferating phase (4-5 days) [18] [19], secondly number of cells will stay almost constant, termed as resting phase or primary plateau phase (5th-13th day), thirdly transitory proliferating phase (13th-15th day) [20] [21] and lastly followed by secondary plateau phase (15th-18th day) [21] [22].

Our drug of interest, 4-amino benzoic benzoyl benzimidazole (4ABBB) or 2-(4-Aminophenyl)-1-benzoyl benzimidazole is a laboratory synthesized derivative of benzimidazole with two nitrogen atoms. It is a slightly yellowish crystalline powder with melting point 157°C and well soluble in organic solvents. It is hypothesized that this derivative might show better antitumor and anticancer activity. Moreover no studies regarding the effect of benzimidazole derivative on EAC cell lines is established. Hence the present study was aimed to figure out whether the systemic administration of 4-amino benzoic benzoyl benzimidazole could modify the cytotoxic effect of EAC cell lines preclinically.

Experimental

Animals

Adult seven-week-old inbred male swiss albino mice (20-25g) were used for the present study. The animals were grouped and housed in standard poly acrylic cages (38×23×10) with 6 animals per cage. They were maintained under controlled laboratory condition such as room temperature (24-28°C-) and relative humidity (40-60%) with 12:12 hour light and dark cycle. All the animals were freely accessed with commercially available mice normal pellet diet and de-mineralized drinking water *ad libitum* throughout the study. All animals were acclimatized for 7 days with the laboratory conditions before commencement of the experiment. The guidelines of the Committee for the purpose of Control and Supervision of Experiments on

Animals (CPCSEA) of the Govt. of India were strictly followed for conducting the preclinical studies [23].

Reagent

O-phenylenediamine and 4 amino benzoic acid were supplied from Central Drug House (India). Acetic acid, Chloroacetic acid, Benzoic acid, Aniline, Benzoyl chloride, Benzyl chloride, Sodium chloride, Acetone and Chloroform were supplied from Rankem (India).

Synthesis of 4-amino benzoic benzoyl benzimidazole

O-phenylene diamine (0.25 mol) and 4-amino benzoic acid (0.34 mol) was heated on a water bath at 100°C for 6-8 h. The reaction mixture was cooled and basified to a pH of 7-8 by using 10% sodium hydroxide solution. The crude benzimidazole was filtered under pressure and washed with ice cold water. The crude product was dissolved in 400 ml of boiling water and 2 g of decolorizing carbon was added and digested for 15 min. The solution was filtered while hot, cooled the filtrate to about 10°C. The pure product was filtered, washed with 25 ml of cold water and dried at 100°C. Benzoyl chloride (0.14 mol) was placed in three necked round bottom flask which was previously fitted with a mechanical stirrer. The flask was immersed in ice cold water and slowly added conc. H₂SO₄ (7.5 ml) down the condenser with slow stirring. After this addition, 2-substituted benzimidazoles (0.028 mol) were added in a portion over a period of 1 h at such a rate that the temperature did not exceed 35°C. After continuous stirring for 12 h, the reaction mixture was poured very slowly over crushed ice with vigorous stirring. The formed product was filtered, washed with cold water and recrystallized from ethanol (scheme is shown in Fig. 1) [24]. The melting point of the compound was found to be 157°C. The drug remains well soluble in 0.2% ethanol, acetone and other organic solvents. The percent yield was found to be 89%. The compound was assessed via chromatographic technique with R_f value 0.80.

Acute toxicity study

The study was done according to OECD guidelines for testing of chemicals 420. Male albino mice were divided into five groups of three animals each. All animals were fasted for 3-4 h (food but not water was withheld). The control group received 0.2% v/v ethanol orally by using a suitable intubation canula. The other groups received 300, 250, 150, 50 mg/kg of 4-ABBB in 0.2% v/v ethanol orally. Doses were prepared shortly prior to administration. At a dose of 300 mg/kg body wt all the animals were died within 24 hrs and at the dose of 50 and 150 mg/kg body wt all the animals remain alive. Consequently at a dose of 250 mg/kg body wt. only half of the animals were died, so this dosage was determined as LD50 of the benzimidazole derivative. The therapeutic dose of 4ABBB was calculated to be 25mg/kg [25].

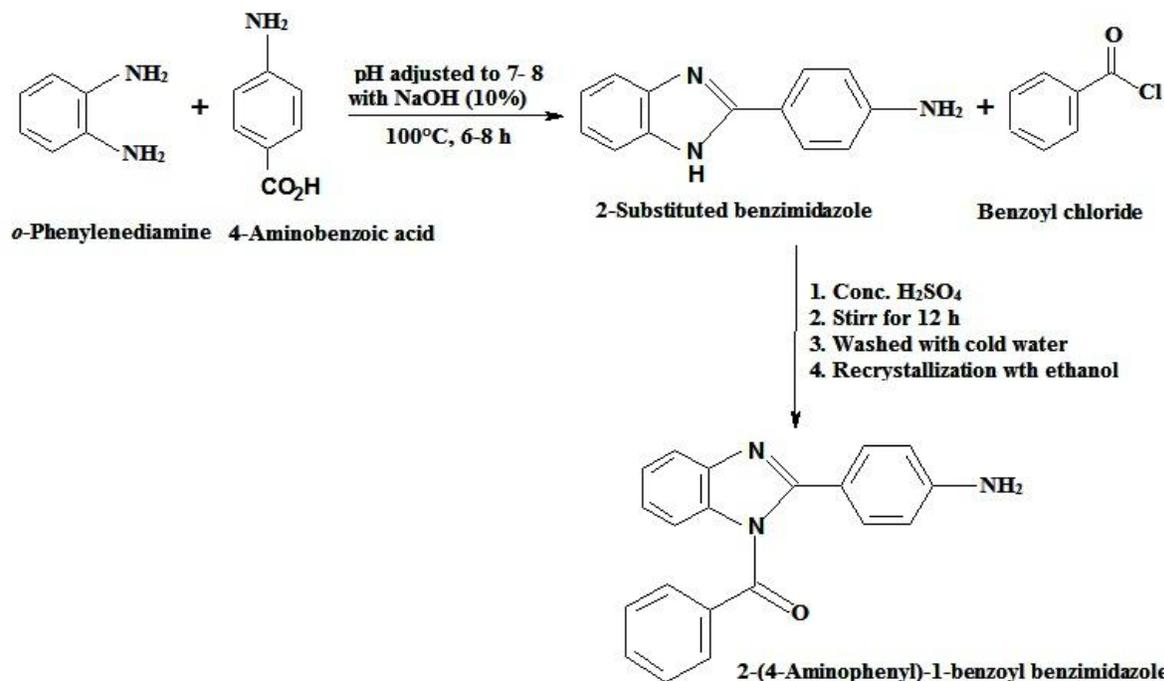


Figure 1. Scheme of reaction for the synthesis of 4-amino benzoic benzoyl benzimidazole (4ABBB) or 2-(4-Aminophenyl)-1-benzoyl benzimidazole.

Establishment and maintenance of EAC cell lines

EAC cell lines were obtained from Jadavpur University, Kolkata, India. The ascitic fluid in a volume of 0.2 ml was collected from intraperitoneal cavity of EAC cell line affected mice at the log phase. The collected ascitic fluid was diluted upto a volume of 10 ml with 0.9% w/v normal saline in a graduated tube. From that fluid, 0.1 ml was transferred to each of the animal employed in this study. After ten days of transplantation, each animal exhibits 2×10^6 tumor cells. Then the EAC animals were divided into various treatment groups [26].

Treatment Protocol

The animals were divided into five groups each containing twenty animals and one group of normal non-EAC animals (control) received 0.2% v/v ethanol. 4-ABBB at a dose of 20 mg/kg (higher dose) and 10 mg/kg (lower dose) were administered intraperitoneal to the different groups of animals ten days. All animals were fasted for 3-4 hrs (food but not water was withheld) prior the drug administration. 5-Fluorouracil at a dose of 20 mg/kg body wt. intraperitoneally was served as reference standard drug. All the drugs were administered for consecutively nine days [26] [27].

Group I: Normal animal received 0.2% v/v ethanol (Normal control).

Group II: EAC animals received 0.2% v/v ethanol (EAC control).

Group III: EAC animals received 4-ABBB (20 mg/kg) in 0.2% v/v ethanol.

Group IV: EAC animals received 4-ABBB (10 mg/kg) in 0.2% v/v ethanol.

Group V: EAC animals received 5-Fluorouracil (5 FU) (20 mg/kg).

Ten animals of each group were sacrificed by cervical dislocation to measure the antitumor and hematological parameters with 18 h of fasting. The rest of the animals were kept with full access of food and water *ad libitum* for determining percentage increase in life span of the tumor host [26].

Determination of tumor volume and weight

At the 10th day, the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it into a graduated centrifuge tube. The tumor weight was measured by taking the weight of the mice before and after the collection of the fluid from peritoneal cavity [26].

Tumor cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer's counting chamber and the numbers of cells in the 64 small squares were counted [26].

Determination of viable and non viable tumor cells

The ascitic fluid was diluted 100 times, 0.4% trypan blue stain was added, waited for 10 minutes and one drop was placed in Neubauer Hemocytometer (MARIE field).

Germany) and the number of viable and non viable cells were counted. The non viable cells (shrunken and crenated) take up the stain and viable cells appear as colorless droplets. Here, cell count = (Number of cells × dilution factor) / (Area × thickness of liquid film) [26] [27]

Hematological Parameters

At the end of the experiment session, few ml of blood was collected from the animals by retro orbital plexus puncture under mild chloroform anesthesia and was employed to determine various parameters.

Determination of hemoglobin concentration (Hb)

A clean hemoglobin tube (Sahli Adams tube) was filled with N/10 HCl solution upto the mark 3g and put it aside. The blood was drawn upto 20 c mm mark by gently sucking on the plastic mouth piece fitted to the rubber tube. Wipe the tip of the pipette with cotton so that no blood left stick to the outside surface. Then the blood was expelled into the Sahli tube containing the HCl solution. Mix the content quickly and gently by shaking the tube before any consequence of clotting. The tube was then kept in a comparator and wait for at least 10 min. During this time the red cells ruptured, liberating the hemoglobin into the solution. The acid reacted with hemoglobin to form Acid hematin which imparted a brown color. After that the tube was taken out from comparator and a few drops of distilled water were added. The content was stirred with glass rod gently and continued to add water drop by drop. The addition continued until the color matched with the standard. Read the upper meniscus and the result was noted in terms of g per 100 ml of blood.

Determination of Red blood cells (RBC) and White blood cells (WBC)

The Neubauer's Chamber was employed for both RBC and WBC count. For RBC count, the blood was drawn upto the mark 0.5 in the RBC pipette, followed by Hayem's fluid (mixture of sodium chloride, sodium sulphate and mercuric chloride) to the mark 101. The bulb contents were mixed thoroughly for one minute. After discarding few drops from the pipette, the counting chamber was charged with the diluted blood. Allow the cell to settle down for one min before starting the count. Under compound microscope (10X), the central one mm square area was focused for counting. Then the cells were counted in the four corner and one middle square- each with sixteen smallest square ie, in 80 squares. The count was expressed in terms of millions per cubic mm [27].

Similarly for the WBC count, the blood was drawn upto the mark 0.5, followed by Turk's fluid (Glacial acetic acid and gentian violet) to the mark 11. Then the contents of the bulb were mixed for two min. The same procedure was followed and the cell was counted in the four groups

of 16 squares each. The total count was expressed in terms of thousands per cubic mm [28].

Determination of packed cell volume (PCV)

0.2 ml of blood was taken in a micro centrifuge tube and the volume was made up to 1.0 ml by normal saline. Add EDTA solution as anticoagulant. The blood sample was centrifuged at 1800 g for 6-8 min and wait for 3 min. The PCV was expressed in terms of percent sedimentation of cells.

Determination of Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC)

It can be calculated from the RBC count in one cubic mm blood and the hemoglobin concentration per cent. Here, $MCH = (Hb \text{ in gm } \%) \times (10/RBC \text{ count in millions per cubic mm})$.

MCHC represents between hemoglobin saturation and cell volume. Here, $MCHC = (Hb \text{ in gm per 100 ml blood}/\text{Volume of packed RBC per 100 ml blood}) \times 100$

Determination of Serum Lactate Dehydrogenase (LDH)

1 ml of blood sample was collected by retro orbital puncture under mild chloroform anesthesia and was centrifuged at 2000g for 8-10 min to separate out the serum. The serum was immediately used to evaluate the LDH level by UV kinetic method using Seimens Diagnostic Kit, India [29] [30].

Percentage increase in life span (ILS) and mean survival time

The effect of 4-ABBB on percentage increases in life span was calculated on the basis of mortality of the experimental mice [27] [31].

$ILS (\%) = [(Mean \text{ survival time of treated group}/Mean \text{ survival time of control group}) - 1] \times 100$

$Mean \text{ survival time (days)} = (\text{first death} + \text{last death})/2$

Statistical Analysis

The statistical analyses were carried out using Graph Pad Prism 5.0 software. All values were presented as Mean ± S.E.M. Comparison between two groups performed using Student t-test. Multiple comparisons between different groups was performed using Analysis of Variance (ANNOVA) followed by Dunetts Multiple comparison Test. Difference level at $P < 0.05$ was considered as statistically significant [26].

Results and Discussion

Results

Effect of 4-ABBB on tumor volume and weight

A significant decrease in tumor volume and tumor weight had been observed in EAC affected animals receiving 4-ABBB both at higher and lower dose as compared with EAC control group ($P < 0.001$, $P < 0.01$). EAC mice treated with standard drug 5 FU also showed a significant improvement both in tumor volume and weight ($P < 0.001$) when compared with EAC control mice as shown in table 1.

Effect of 4-ABBB on tumor cell count

Table 1 showed a significant decrease in total tumor cell count in 4-ABBB treated EAC mice both at higher and lower dose when compared with EAC control group ($P < 0.001$, $P < 0.01$). The standard drug 5 FU also showed a significant improvement in EAC affected group ($P < 0.001$).

Effect of 4-ABBB on viable and non-viable tumor cell count

Table 1 showed a significant decrease in viable tumor cell count and increase in non-viable tumor cell count in 4-ABBB treated EAC mice both at higher and lower dose when compared with EAC control group ($P < 0.01$, $P < 0.001$). The standard drug 5 FU also showed a significant improvement in EAC affected group ($P < 0.01$, $P < 0.05$).

Effect of 4-ABBB on hematological parameters

Table 2a showed a significant improvement in hematological parameters (hemoglobin, RBCs, WBCs, PCV) in animals receiving 4-ABBB both at higher and lower dose as compared with EAC control animals ($P < 0.01$, $P < 0.05$). Some other parameters such as MCH, MCHC and serum LDH were also assessed in all the groups and a significant improvement was found 4-ABBB treated groups as shown in table 2b ($P < 0.001$, $P < 0.01$, $P < 0.05$). The standard drug 5 FU showed a significant improvement in EAC affected group ($P < 0.001$, $P < 0.01$, $P < 0.05$).

Table 1. Effect of 4-ABBB on tumor volume, tumor weight, tumor cell count, viable tumor cell count and non-viable tumor cell count in EAC affected mice

Group	Treatment	Tumor volume (ml)	Tumor weight (g)	Tumor cell count ($\times 10^7$ /ml)	Viable tumor cell count ($\times 10^7$ /ml)	Non-viable tumor cell count ($\times 10^7$ /ml)
I	Normal Control (0.2% ethanol)	-	-	-	-	-
II	EAC control	3.600 \pm 0.11	4.067 \pm 0.088	6.057 \pm 0.626	4.34 \pm 0.30	1.717 \pm 0.04
III	EAC + 4-ABBB (20mg/kg i.p)	0.800 \pm 0.05 ^c	2.100 \pm 0.052 ^c	5.717 \pm 0.332 ^c	2.38 \pm 0.29 ^b	3.337 \pm 0.23 ^c
IV	EAC + 4-ABBB (10mg/kg i.p)	1.767 \pm 0.088 ^b	3.100 \pm 0.053 ^b	5.800 \pm 0.301 ^b	3.46 \pm 0.25 ^a	2.340 \pm 0.31 ^a
V	EAC + 5-FU (20mg/kg i.p)	0.526 \pm 0.017 ^c	2.033 \pm 0.089 ^c	5.600 \pm 0.103 ^c	1.78 \pm 0.10 ^b	3.820 \pm 0.30 ^c

The statistical significance of difference between means was calculated by ANOVA followed by t-test for unpaired comparison. N=10 Values are expressed as Mean \pm S.E.M, ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$



Figure 2. Effect of 4 ABBB on tumor cells parameter of EAC treated mice. Tumor volume (ml) (1A), Tumor weight (g) (1B), Viable tumor cell count ($\times 10^7$ /ml) (1C), Non-viable tumor cell count ($\times 10^7$ /ml) (1D).

Table 2a. Effect of 4-ABBB on hematological parameters in EAC affected mice

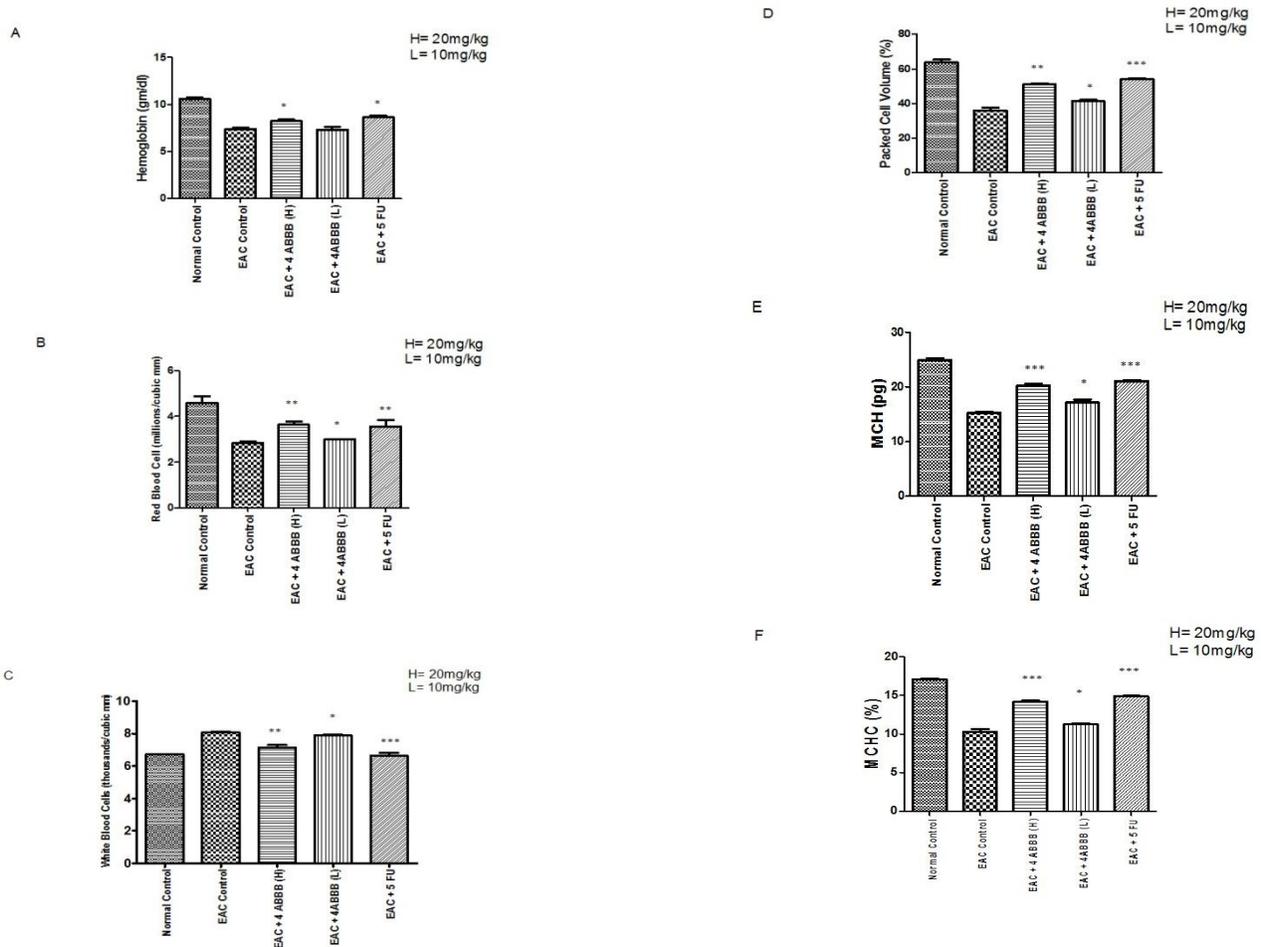
Group	Treatment	Hemoglobin (g/dl)	RBC count (millions/cubic mm)	WBC count (thousands/cubic mm)	PCV (%)
I	Normal Control (0.2% ethanol)	10.57±0.20	4.583±0.29	6.719±0.01	63.67±1.85
II	EAC control	7.377±0.19	2.830±0.68	8.075±0.037	36.00±1.7
III	EAC + 4-ABBB (20mg/kg i.p)	8.233±0.14 ^a	3.633±0.14 ^b	7.147±0.14 ^b	51.00±0.5 ^b
IV	EAC + 4-ABBB (10mg/kg i.p)	7.33±0.28	2.99±0.008 ^a	7.888±0.03 ^a	41.33±0.88 ^a
V	EAC + 5-FU (20mg/kg i.p)	8.633±0.21 ^a	3.56±0.28 ^b	6.623±0.19 ^c	54.00±0.57 ^c

The statistical significance of difference between means was calculated by ANOVA followed by t-test for unpaired comparison. N=10
 Values are expressed as Mean ± S.E.M, ^aP< 0.05, ^bP< 0.01, ^cP< 0.001

Table 2b. Effect of 4-ABBB on hematological parameters in EAC affected mice

Group	Treatment	MCH (pg)	MCHC (%)	Serum LDH (U/L)
I	Normal Control (0.2% ethanol)	24.93±0.34	17.10±0.05	55.96±0.44
II	EAC control	15.27±0.17	10.33±0.33	71.96±0.85
III	EAC + 4-ABBB (20mg/kg i.p)	20.20±0.41 ^c	14.17±0.16 ^c	61.77±0.75 ^c
IV	EAC + 4-ABBB (10mg/kg i.p)	17.12±0.61 ^a	11.23±0.14 ^a	66.43±0.30 ^b
V	EAC + 5-FU (20mg/kg i.p)	21.07±0.12 ^c	14.90±0.05 ^c	60.34±0.34 ^c

The statistical significance of difference between means was calculated by ANOVA followed by t-test for unpaired comparison. N=10
 Values are expressed as Mean ± S.E.M, ^aP< 0.05, ^bP< 0.01, ^cP< 0.001



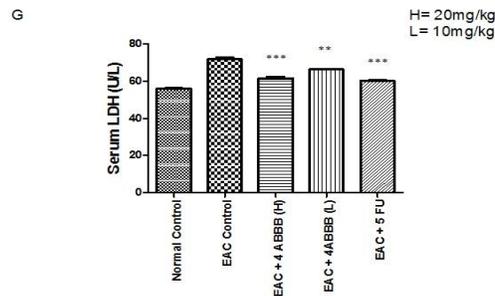


Figure 3. Effect of 4-ABBB on hematological and serum parameters of EAC treated mice. Hemoglobin (gm/dl) (2A), Red blood cell (millions/cubic mm) (2B), White blood cells (thousands/cubic mm) (2C), Packed cell volume (%) (2D), MCH (pg) (2E), MCHC (%) (2F), Serum LDH (U/L) (2G).

Table 3. Effect of 4-ABBB on percentage increase in life span (ILS) and mean survival time

Group	Treatment	ILS (%)	Mean survival time (days)
I	Normal Control (0.2% ethanol)	-	-
II	EAC control	00.00	23.5±0.96
III	EAC + 4-ABBB (20mg/kg i.p)	50.02±0.20 ^c	38.5±0.23 ^c
IV	EAC + 4-ABBB (10mg/kg i.p)	29.08±0.03 ^c	30.5±0.21 ^b
V	EAC + 5-FU (20mg/kg i.p)	90.12±0.20 ^c	46.5±0.12 ^c

The statistical significance of difference between means was calculated by ANOVA followed by t-test for unpaired comparison. N=10
Values are expressed as Mean ± S.E.M, ^b P< 0.01, ^c P< 0.001

Effect of 4-ABBB on percentage increase in life-span and mean survival time

Table 3 showed a significant increase in life span and mean survival time in groups receiving 4-ABBB both at higher and lower dose as compared with EAC control animals (P<0.01, P<0.001). Groups receiving 5-FU as standard drug also showed a significant improvement in life span and mean survival time (P<0.001).

Discussion

Ehrlich ascites carcinoma (EAC) is an undifferentiated carcinoma characterized by hyperdiploidy and highly transplantable capability. They do not have tumor specific transplantation antigen (TSTA) [32]. Generally, repetitive passages increase tumor virulence via rapid proliferation. Ascites fluid is grayish white in color and contains about 10 millions of neoplastic cells in 0.1 cc [33]. EAC induced carcinoma has a close resemblance like human tumors that are very sensitive to chemotherapy. Though various scientists reported that plant extracts were found to be effective against the rapid proliferating cells, still no mechanistic approach had been revealed. Hence the study aimed to assess the role of benzimidazole derivative in EAC induced animals.

In the present work, EAC when induced in various groups showed rapid accumulation of intraperitoneal fluid within ten days. This rapid proliferation exerted high hydrostatic pressure on the wall of the peritoneum which ultimately decreased the life span of host animals. After administration of 4-ABBB in infected mice, there is marked decrease in tumor cell volume and weight which may be due to up-regulation of humoral factors and diminished rate of cell division. The drug also shows the

effect in a dose-dependent manner. Moreover, the viable tumor cell count is decreased at 10th day by the drug at higher dose (20mg/kg body weight) which established the fact that the drug may have a potential anti-tumor effect. This suggests that the drug might be absorbed by these tumor cells in peritoneal cavity and involved in lysis of these cells by cytotoxic mechanisms [34]. It can be hypothesized that as the drug contains three nitrogen atoms attached with benzene ring at the meta and para positions, hence it may exert its beneficial effects by repairing the purine and pyrimidine bases of DNA via interacting with the nitrogen atoms [35] [36]. Standard drug 5-FU also showed significant result as anti-tumor activity probably by inhibiting thymidylate synthase and blocking the conversion of deoxyuridilic acid to deoxythymidylilic acid [37] [38].

The establishment of the drug as a potent anticancer agent can be established by the ability of the decrease of WBC and prolongation of the life span [39]. Generally during chemotherapy, a rapid decrease in RBC has been noticed which results in anemia. The EAC induced mice showed a decrease in RBC count, hemoglobin level and percent packed cell volume and increase in WBC count which establish the fact that the animals affected by ascites carcinoma. Administration of 4-ABBB at different dose levels in mice recovered the hematological conditions significantly. As EAC induced carcinoma is linked with the cytokine and nuclear factor α expression, the increase in WBC count is common increased in any type of cancer. The drug significantly decreased the WBC count probably by down-regulating the cytokine expression and reduced prevalence of infections caused due to EAC cells. The reduction of tumor cell count by the drug also

imparts the fact that there is a connection between the improvements in the condition of the EAC affected animals. In carcinogenic stage, RBC count is also affected due to impaired erythropoiesis in bone marrow of the host animals. Moreover, restoration in RBC count, hemoglobin level and percent PCV in 4-ABBB treated mice proves that the drug might have an anti-cancer potentiality. Improvement in mean survival time and percent increase in life span are also noticed by the animals receiving the drug which suggests that the drug might heal the carcinoma injury and increased the life expectancy.

Serum LDH is an important marker regarding cellular injury or necrosis. Generally LDH_A is highly expressed in malignant cells [40] [41]. Generally LDH_A is involved in conversion of pyruvate to lactate and acts as key source of anaerobic glycolysis. It is found to be increased in many types of cancer which is directly linked with rapid growth of tumor cells and their invasion in host cells. Hence the serum LDH level is an important marker to determine the severity of ascitis carcinoma in mice. 4-ABBB treated mice showed a significant restoration of serum LDH level which further proves that the drug might possess an anti-cancer potential.

From the above discussions, it is quite clear that the benzimidazole derivative, 4-ABBB which has structural similarity with DNA bases can be cytotoxic either by alkylation with the base pairs of malignant cells DNA or decreasing the rate of protein synthesis or reducing glutathione concentration or inhibiting T cells responses [42] [43] [44]. The reference drug 5-FU is a well established potent anticancer drug which acts via its active metabolite fluorodeoxyuridine monophosphate. This active metabolite binds covalently with the nucleotide binding site of thymidylate synthase. As a result, the catalysis of deoxyuridine monophosphate into deoxythymidine monophosphate will be inhibited and results in imbalance of deoxynucleotide pool. This imbalance indirectly increases levels of deoxyuridine triphosphate that causes DNA damage of the malignant and host cells [45] [46].

Conclusion

Taken together these results come to a conclusion that the drug 4-ABBB has a promising role as antitumor and anticancer agent in EAC induced albino mice. The evidence from the results further proves that the drug can increase the life span of host cells. The study tries to figure out the mechanistic approach of the drug towards its cytotoxic effect. But various parameters related to malignant cell death are still to be explored as future prospects. Further preclinical and clinical studies can be done to completely evaluate the exact mechanism of action of the 4-ABBB and its adverse drug reactions, dose

level, interactions etc so that the drug can be used as therapy in carcinomas.

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