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ANTI TUMOUR ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *BOERHAAVIA DIFFUSA* LINN AGAINST DALTON ASCITIC LYMPHOMA& EHRLICH ASCITIC CARCINOMA IN MICE

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ABSTRACT

The present study aims to evaluate the antitumor activity of hydro alcoholic extract of whole plant of *Boerhaavia diffusa* linn on Dalton Ascitic Lymphoma (DAL) and Ehrlich Ascitic carcinoma (EAC) in Swiss Albino mice model.The tumor was induced in mice by intraperitoneal injection of DAL ($1X10^6$cells/mouse). Hydro alcoholic extract of *Boerhaavia diffusa* linn (HAEBD) was administered to the experimental animals at a dose of 200mg/kg & 400mg/kg after 24 h of DLA induced tumor inoculation. The activity was assessed by increase in life span, average decrease in body weight, tumor weight, tumor volume, viable cell count, packed cell volume, hematological and biochemical parameters.The potency of the extract was compared with standard 5-flurouracil (20mg/kg I.P).HAEBD treatment showed significant increase in the life span, decrease the cancer cell count and tumor weight in the DLA tumor induced mice. The results showed decrease in tumor volume and cell viability. The antitumor effect of HAEBD was also evaluated by assessing hematological parameters and biochemical parameters. Hematological parameters like red blood cell count, hemoglobin content and platelets cell values were restored to near normal level by HAEBD in tumor induced mice. In addition, HAEBD brought back the altered levels of liver enzyme towards normal value. Histopathological studies also reveals the normal architecture of liver tissues.In conclusion, the present study results revealed that Hydro alcoholic extract of *Boerhaavia diffusa* linn possessed significant antitumor activity against DAL and EAC models in mice.

Keywords: *Boerhaavia diffusa linn*, Dalton's Ascitic lymphoma, Erhlich Ascitic Carcinoma, 5-Fluoro uracil, Tumour volume, Life span

INTRODUCTION

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year [1].It is a type of hyper proliferative disorder that involves transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis. In recent times, the

trend in cancer research is shifting towards identifying new medicines from natural resources for the management of cancer. Therefore there is a constant demand to develop new, effective, and affordable anticancer drugs [2].Though Chemotherapy is now being used as a standard treatment method drugs obtained from medicinal plants play a crucial role in the treatment of cancer [3]. Moreover, current anti- cancer regimens are frequently associated with significant levels of toxicity and the emergence of drug resistance. Plant derived from natural products such as flavonoids, terpenoids, and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [4,5].In the treatment of cancer, many herbal remedies have been employed in various medical systems for the treatment and management of different diseases. *Boerhaavia diffusa* Linn is one of the renowned medicinal plants used to treat large number of human ailments. *Boerhaavia diffusa* Linn, Nyctaginaceae, commonly known as 'Punarnava' in the Indian system of medicine. It is a perennial creeping herb found throughout the waste land of India. The whole plant of *B. diffusa* has been employed for the treatment of various disorders like liver disorders, gastrointestinal disorders, and heart diseases. The roots are reputed to be diuretic and laxative and are given for the treatment of anasarca, ascites and jaundice [6]. The plant has also been screened for anti-inflammatory, antimicrobial, immunosuppressive, hepatoprotective, antitumorogenic, anti-leprotic and anti-asthmatic activities [7-10]. *Boerhaavia diffusa* Linn contains large number of phytoconstituents, like flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins and glycoproteins [11,12].

MATERIALS AND METHOD

Collection and Authentication of Plant

Whole plant of *Boerhaavia diffusa* Linn was collected from local traders, Tamil Nadu. The plant material was taxonomically identified and authenticated by Dr.Stephan, Botanist, American college, Madurai and the voucher specimens (KMCP/GN/BD/0290) were retained in the Institute for future reference. The whole plant of *Boerhaavia diffusa* linn was dried in shade, milled into coarse powder by a mechanical grinder and stored in closed vessel for further use.

Experimental animals

Male Swiss albino mice (20-25 gm) were produced from animal experimental laboratory, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp 25±2°C) and 12 hrs dark /light cycle with standard laboratory diet and water *ad libitum.* The experimental protocol was approved by Institutional animal ethical committee (Reg.no.661/02/C/CPCSEA&19/07/2002).App.no.G.Nalini/TNMGRMU/Ph.D/IAEC/KMCP112/2014-15). CPCSEA guidelines were adhered during the maintenance and experiment. As per the standard practice, the mice were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house [13].

Plant crude extract

About 500gm of air dried coarse powder of *Boerhaavia diffusa* linn were soaked in the extractor and macerated for 30 hrs with petroleum ether and then it is refluxed successfully with chloroform; benzene then extracted with alcohol and water (70:30) by continuous hot percolation method using soxhlet apparatus for 72 hrs separately. Hydro alcoholic extracted of *Boerhaavia diffusa* linn was filtered and concentrated in vacuum using rotary flask evaporator under reduced pressure. Then the extract of *Boerhaavia diffusa* linn concentrated to brownish residue stored in air tight container.

Induction of Tumour using DAL & EAC cells

Dalton's Lymphoma ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cells were obtained courtesy of Amla Cancer Research center, Thrissur, Kerala, India. The cells were maintained in vivo in swiss albino mice by intra peritoneal transplantation. While transforming the tumour cells to the grouped animal, the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell count was done and further dilutions were made, so that total cells should be $1x10^6$ cells /ml/mouse. This volume was given intraperitoneally and the tumour was allowed to grow in the mice for a minimum of seven days before starting the study [15-18].

Treatment Protocol

Swiss Albino mice were divided in to five group of six each. All the animals in four groups (G2-G4) were injected with DLA cells (1×10^6 cells per mouse) intraperitoneally, except the normal control group. After the inoculation the groups were treated as given below.

Group 1 served as Control.

Group 2 served as Tumor control. Group 1 and 2 receives normal diet and Water.

Group 3 served as the positive control, was treated with injection 5-fluorouracil at 20 mg/kg body weight, Intraperitoneally. [19]

Group 4 served as treatment control, which was treated with Hydro alcoholic extract of *Boerhaavia diffusa* Linn (HAEBD) at a dose of 200 mg/kg body weight, given through orally.

Group 5 served as treatment control which was treated with Hydroalcoholic extract of *Boerhaavia diffusa* linn (HAEBD) at a dose of 400 mg/kg of body weight, given through orally. In this study, drug treatment was given after 24 h of inoculation, once daily for 14 days. On day 15, the following derived parameters were estimated [20].

Derived parameters

Body weight

All the mice were weighed, from day1 to 14^{th} day of the study. Average increase in body weight on the 15^{th} day was determined.

Percentage increase in life span (ILS)

The effect of HAEBD on tumor growth was monitored by recording the mortality daily for 6 weeks and percentage increase in life span (ILS) was calculated [21].

$$\%\mathbf{ILS} = \frac{Life\ span of\ treated\ group}{Life\ span of\ control\ group} - 1x100$$

Effect of HAEBD on Survival Time

% ILS was calculated by the following formula

Increase in lifespan = [(T - C) / C] x 100

Where T = number of days the treated animal survived.

C = number of days control animals survived.

Evaluation of Clinical Parameters

Cancer cell count

The fluid (0.1ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold Normal saline or sterile Phosphate Buffer Solution and 0.1 ml of

tryphan blue (0.1 mg/ml) and total numbers of the living cells were counted using heamocytometer [22-27]

$$\text{Cell count} = \frac{\text{No of cells Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

Hematological parameters

The effect of HAEBD on hematological parameters was studied in the mice of all groups. In this study, the drug treatment was given 24 h after inoculation, once daily for 14 days. On day 14th after the last dose, all mice from each group were sacrificed by euthanasia. Blood was withdrawn from each mouse by retro orbital puncture method and Hematological parameters, biological parameters enzyme and lipid profile were analyzed [27-28].

Serum enzyme and lipid profile

The effect of HAEBD on serum enzyme and lipid profile including total cholesterol, TG. AST, ALT, ALP was evaluated. All biochemical investigations were done by using COBAS MIRA PLUS-S Auto analyzer from Roche Switzerland. Hematological test are carried out in COBAS MICROS OT 18 from Roche. Newly added Hi-Tech instruments MAX MAT used for an auto analyzer for all biochemistry investigations in blood sample [29-32].

Effect of HAEBD on Solid Tumor

Mice were divided into three groups (n=6). Tumor cells ($1x10^6$ cells/mice) were injected into the right limb (thigh) of all the animals intramuscularly. The mice of Group I were tumor control. Group II and Group III received HAEBD (200&400mg/kg) orally for 5 alternative days. Tumor mass was measured from the 11th day of tumor induction. Diameter of the tumor was measured on every 5th day for a period of 30 days using Vernier calipers and the volume of the tumor mass was calculated using the formula $V= 4/3\ \pi\ r^2$ where r is the mean of r^1 and r^2 which are two independent radii of the tumor mass.

Histopathological examination

A piece of liver, samples were fixed in 10% formalin for Histopathological examination. The thin sections were cut and then stained by Haematoxylin and Eosin and observed under light microscope [33].

Statistical analysis

The results were expressed as Mean± S.E.M and analyzed with one way analysis of variance (ANOVA) between the groups and followed by Newman keul's multiple range tests. Probability values $P< 0.05$ were considered as significant.

RESULTS

Effect of HAEBD on survival time & Tumor Growth

The effect of HAEBD on survival of DAL tumor bearing mice were shown in Table1.In the DLA tumor control group, the average life span of animal was found to be 46%. Whereas HAEBD at the dose of 200mg/kg and 400 mg/kg significantly increase the life span to 80% and 86% respectively. However the average life span of 5- FU treatment was found to be 90%, indicating its potent antitumor nature. The antitumor nature of HAEBD was evidenced by the significant reduction in percent increase in body weight of animal treated with HAEBD at the dose of 200mg/kg and 400 mg/kg body weight when compared to DLA tumor bearing mice. It was also supported by the

significant reduction in packed cell volume and viable tumor cell count in both the extent of treatment when compared to the DLA tumor control. (Table No: 1).

Effect of HAEBD on Hematological Parameters

In Hematological parameter the total WBC count was significantly increased, where as RBC, Hb, and Platelets were decreased in the DLA control group compared to the normal control group. Treatment with HAEBD at the dose (200mg/kg and 400 mg/kg) significantly increases the Hb content, RBC, Platelets and significantly decreased the WBC count to about near normal values. Also HAEBD significantly reduced PCV content to 21.26±1.45% and 20.55±1.38%. All these results suggest the anticancer nature of the HAEBD.

Effect of HAEBD on Biochemical Parameters

The inoculation of DLA cells caused significantly increase in the level of total Cholesterol, Aspartate amino Transferase, Alanine amino Transferase, Alkaline Phosphatase in the tumor control animals (G_2), when compared to the normal group. Treatment with HAEBD at the dose of 200mg/kg and 400 mg/kg body weight significantly reversed these changes towards the normal level. (Table No.3) All these value were found to be significant.

Effect of HAEBD on EAC Induced Solid Tumor

In the tumor control animals the solid tumor volume induced by EAC cells was found to be significantly increased from day 0 to day 30. However, the tumor volume was significantly decreased in animal treated with HAEBD at 200mg/kg and 400 mg/kg body weight. A significant reduction in solid tumor volume was seen from day 15 to the end of the experiment. About 27% and 30% reduction in tumor volume was observed with HAEBD treatment respectively on the day 30 of the experiment (Table No.4).

Table No.1 Effect of HAEBD on the life span, body weight and cancer cell count of tumor induced mice.

Treatment	Number of animals	% ILS Life span	Increase in Body weight grams	Cancer cell count ml X 10^6
G_1	6	>>30 days	2.22±0.68	-
G_2	6	46%	$9.44±0.86^{a**}$	$2.75±0.40^{a**}$
G_3	6	90%	$5.66±0.42^{b**}$	$1.30±0.40^{b**}$
G_4	6	80%	$6.22±0.60^{b**}$	$1.65±0.45^{b**}$
G_5	6	86%	$5.80±0.80^{b**}$	$1.50±0.30^{b*}$

G_1 – Normal Control, G_2 – Cancer Control, G_3 – Positive control, G_4 – Treatment control (HAEBD 200mg/kg) G5 – Treatment control (HAEBD 400mg/kg)

All values are expressed as mean ± SEM for 6 animals in each group.

**a – Values are significantly different from control (G_1) at $P < 0.001$

**b – Values are significantly different from cancer control (G_2) at $P < 0.001$

Table No. 2. Effect of HAEBD on hematological parameters

Treatment	Total WBC Cells /mlx10^3	RBC Count Mill/cumm	Hb Gm/dl	PCV %	Platelets Lakhs/cumm
G1	10.85 ±0.86	4.68±0.46	12.05 ±0.45	15.80±1.55	3.35±0.36
G2	14.05 ±1.42^{a**}	2.30±0.15^{a**}	7.30 ±0.30	31.60±2.60^{a**}	1.75±0.18^{a**}
G3	11.42 ±0.95^{b**}	3.95±0.32^{b**}	11.6 ±0.35^{b**}	18.30±1.10^{b**}	2.80±0.22^{b**}
G4	12.80±1.02^{b**}	3.25±0.22^{b**}	10.05±0.18^{b**}	21.26±1.45^{b**}	2.15±0.12^{b**}
G5	12.10 ±0.98^{b**}	3.60±0.28^{b**}	11.05±0.26^{b**}	20.55±1.38^{b**}	2.45 ±0.16^{b**}

G_1 – Normal Control, G_2 – Toxic Control, G_3 – Positive control, G_4 – Treatment control (HAEBD 200mg/kg), G5 – Treatment control (HAEBD 400mg/kg)

All values are expressed as mean ± SEM for 6 animals in each group.

**a – Values are significantly different from normal control (G_1) at $P < 0.001$

**b – Values are significantly different from toxic control (G_2) at $P < 0.001$

Table No.3. Effect of HAEBD on Serum Enzymes and Lipid Proteins

Treatment	Cholesterol (mg/dl)	TGL (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G_1	110.15±1.66	125.5±2.40	38.50 ±1.25	33.40 ±1.26	126.20 ±1.20
G_2	145.65±2.45^{a**}	210.20±4.25^{a**}	88.10±1.85^{a**}	58.20±1.75^{a**}	225.30±3.35^{a**}
G_3	116.55±0.92^{b**}	162.85±3.15^{b**}	56.20 ±1.68^{b**}	42.30±1.63^{b**}	160.40±1.45^{b**}
G_4	134.52±1.50^{b**}	172.95±3.22^{b**}	71.80 ±2.25^{b**}	50.20±1.88^{b**}	185.30±1.68^{b**}
G_5	128.60±1.42^{b**}	166.30±2.78^{b**}	64.40±1.91^{b**}	46.40 ±1.70^{b**}	178.52±1.55^{b**}

G1 – Normal Control, G2 – Toxic Control, G3 – Positive control, G4 – Treatment control (HAEBD 200mg/kg), G5 – Treatment control (HAEBD 400mg/kg)

All values are expressed as mean ± SEM for 6 animals in each group.

**a – Values are significantly different from normal control (G_1) at $P < 0.001$

**b – Values are significantly different from toxic control (G_2) at $P < 0.001$

Table No. 4 Effect of HAEBD on Solid Tumor Volume

Treatment	Dose	Solid tumor Volume (ml)			
		15th Day	20th Day	25th Day	30th Day
G_1	2 ml/kg Saline	2.65±0.16	3.60±0.16	4.70±0.20	5.80±0.16
G_2	200 mg/kg HAEBD	2.40±0.18	3.40±0.25	3.60±0.18	4.20±0.08**a
G_3	400 mg/kg HAEBD	2.68±0.20	3.32±0.18	3.40±0.15	4.10±0.05**a

G1 – Normal Control, G2 – Treatment control (HAEBD 200mg/kg),

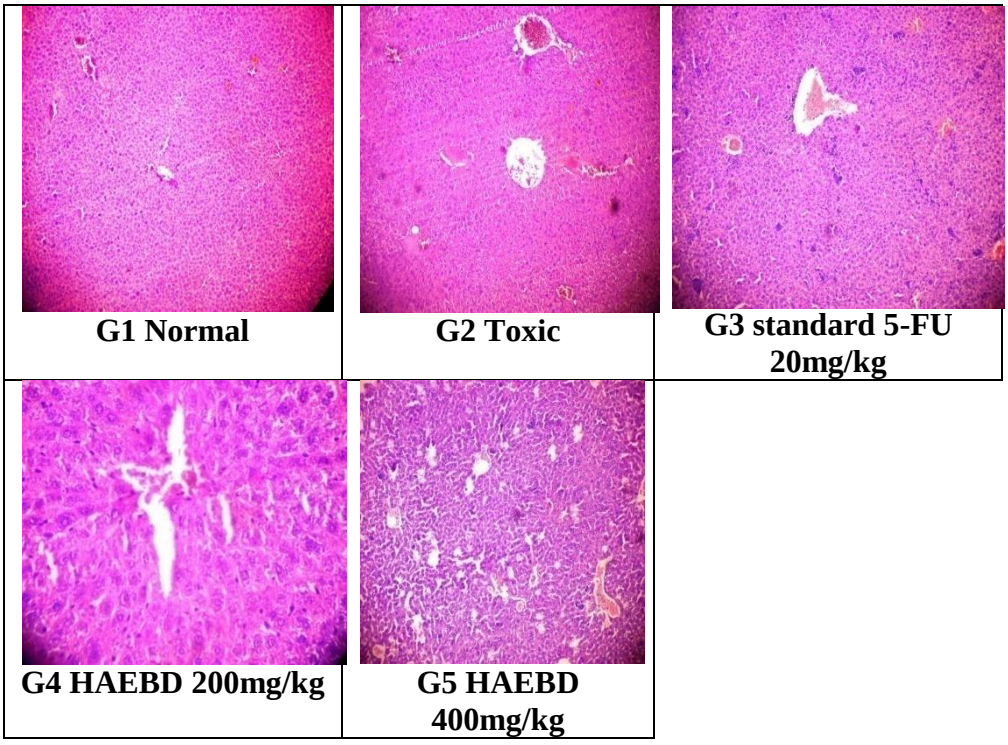
G3 – Treatment control (HAEBD 400mg/kg)

All values are expressed as mean ± SEM for 6 animals in each group.

**a – Values are significantly different from cancer control (G_1) at $P < 0.01$

HISTOPATHOLOGY

G1-Section show structure of liver with sheets of hepatocytes seperated by sinusoids cartial vein & portal tract appear normal.**G2**- Section shows structure of liver presented hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, Kupffer cell proliferation, hepatocyte diffuse necrosis and mononuclear infiltrate.**G3**-Section show structure of liver presented mild hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, No Kupffer cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.**G4**- Section show structure of liver presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less Kupffer cell proliferation, moderate hepatocyte diffuse necrosis and mononuclear infiltrate.**G5**- Section show structure of liver presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less Kupffer cell proliferation, moderate hepatocyte diffuse necrosis and mononuclear infiltrate.



DISCUSSION

In the present study inoculation of DAL cells in mice produced an enormous increase in the tumour cell count which indicated that there is progression of cancer in mice. Treatment with Hydro alcoholic extract of *Boerhaavia diffusa* Linn inhibited the viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals. Ascitic fluid is the direct nutritional source for tumor cells and rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells.It may be concluded that the Hydro alcoholic extract of *Boerhaavia diffusa* Linn by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DAL bearing mice. Thus, Hydro alcoholic extract of *Boerhaavia diffusa* Linn has antitumor activity against DAL bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelo suppression and anemia. [34] The anemia encountered in tumor bearing mice its mainly due to

reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic condition.[35] In tumor bearing mice, it was found that there was increase in WBC count, and decrease in Hb content and RBC count. Treatment with Hydro alcoholic extract of *Boerhaavia diffusa* Linn brought back the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels. This clearly indicates that Hydro alcoholic extract of *Boerhaavia diffusa* Linn possess protective action on the hemopoietic system.

In the present study, the biochemical examination of DLA inoculated animals showed marked changes indicating the toxic effect of the tumor. It was reported that the presence of tumor in the human body or in the experimental animals is known to affect may function of the liver. An abnormal lipid profile has been associated with cancer. Therefore liver enzymes level markedly increased in tumor bearing mice. The significantly elevated level of total cholesterol, TG, AST, ALT, ALP in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by HAEBD treatments indicates a protective effect on liver enzymes.

Presence of Boeravinone, Punarnavine, Punarnavoside, a phenolic glycoside phytosterols, triterpenes, flavonoids and glycosides shown to possess antimalignant & antitumour effects. Flavonoids show chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis. [36]

CONCLUSION

In the conclusion, The Hydro alcoholic extract of the plant *Boerhaavia diffusa* Linn possess significant anti-cancer activity against DLA and EAC induced in mice. This herb was effective in inhibiting the tumor growth in ascitic and solid tumor models. The biochemical and Histopathological studies also supported its antitumor properties. However, further investigations required to isolate and identify the active constituents responsible for its anti-cancer activity.

Conflicts of Interest

No conflict of interest was declared.

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