

Effect of leaf extracts of *Rhynchosia Beddomei* on Ehrlich Ascites Carcinoma in mice

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Abstract

The leaf extracts of *Rhynchosia bedomei* exhibited antitumor activities against Ehrlich Ascites Carcinoma in Swiss albino mice. Animals were treated with EAC cell lines (at a dose of 2×10^6 cells) and then after 24hrs, ethyl acetate and alcohol extract at a dose of 100mg and 200mg/kg, were administered for a period of 14 days. Later the mice were sacrificed for studying of antitumor activity. The decrement of tumor volume, packed cell volume and viable cell count were observed in both ethyl acetate and alcohol extract treated animals and compared with 5-fluorouracil treated and only EAC tumor bearing mice. Treatment with test compounds increased the survival time and restores the normal peritoneal cell count. Hematological parameters, PCV which were altered in EAC treated animals, were re-established considerably. Thus, the study is an attempt to evaluate the preventive and curative role of *Rhynchosia bedomei* in tumor bearing mice.

Key words:

Ehrlich Ascites Carcinoma, *Rhynchosia bedomei*

How to Cite this Paper:

N. B. Sridharamurthy*, R. Yogananda, Dr. Riyaz Mahamood "Effect of leaf extracts of *Rhynchosia Beddomei* on Ehrlich ascites carcinoma in mice", Int. J. Drug Dev. & Res., Oct-Dec 2011, 3(4): 225-236

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Article History:-----

Date of Submission: 24-08-2011

Date of Acceptance: 12-10-2011

Conflict of Interest: NIL

Source of Support: NONE

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INTRODUCTION

Cancer is a group of diseases with similar characteristics, which can occur in all living cells in the body and different cancer types have different natural history. The myths that cancer affects people mostly in the developed countries is broken by the

fact that, the 10 million new cancer cases seen each year worldwide, nearly 5.5 million are in the less developed countries. Cancer is the second most common cause of death in the developed world and a similar trend has emerged in the developing countries too. Cancer prevalence in India is estimated to be around 2.5 million, with 8, 00,000 new cases and 5, 50, 000 deaths occurring each year due this disease [1].

Cancer is the leading cause of mortality and most of the synthetic chemotherapeutic agents have been reported to exhibit severe normal tissue toxicity, accompanied by undesirable side effects. More over these drugs are highly expensive mutagenic and carcinogenic. Therefore the substitute of the conventional chemotherapeutic agents to control the high mortality rate are needed which will be highly effective at non toxic doses and inexpensive and accessible to general people. This can be achieved by in-depth research and continuous screening of new molecules or natural agents. The natural medicines have played a great role to treat various disorders in humans including cancer [2].

Recent surveys indicate that plants, vegetables and herbs as folk and traditional medicine have been accepted widely as major resources of chemo preventive agents. of which *Rhynchosia beddomei Baker*, is plant of natural origin belongs to the family Fabaceae is a rare and endemic plant restricted to Seshachalam (Gogarbham hills) of Eastern Ghats of Andhra Pradesh, India. Bakshu *et al.*, (2001), studied the phytochemical properties of the leaves and reported the presence of alkaloids, indole alkaloids, anthracene glycosides, anthraquinones, carotenoids, coumarins, dihydrochalcones, fatty acids, flavonoids, flavones, flavonols, steroids and triterpenoids[3].

As per the review of literature it is observed that the plant selected contains mixture of flavonoids as an active constituent as reported by Bakshu *et al.*, [3]. The flavonoids are polyphenolic compounds found

as integral components of the human diet. They are universally present as constituents of flowering plants, particularly of food plants. It is also observed that *in vitro* work has concentrated on the direct and indirect actions of flavonoids on tumor cells, and has found a variety of anticancer effects such as cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and tumor invasive behavior. Furthermore, some studies have reported the impairment of *in vivo* angiogenesis by dietary flavonoids. Experimental animal studies indicate that certain dietary flavonoids possess antitumor activity. The hydroxylation pattern of the B ring of the flavones and flavonols, such as luteolin and quercetin, seems to critically influence their activities, especially the inhibition of protein kinase activity and antiproliferation. Certain dietary flavonols and flavones targeting cell surface signal transduction enzymes, such as protein tyrosine and focal adhesion kinases, and the processes of angiogenesis appear to be promising as anticancer agents. Further *in vivo* studies of these bioactive constituents are deemed necessary in order to develop flavonoid-based anticancer strategies [4, 5].

Review of literature also provides the information about the uses of plant in traditional medicine as the leaves of the plant are used for wounds, cuts, boils and rheumatic pains by Adivasi tribes (Sugali, Yanadi, Chenchu, Nakkala, Erukala) inhabiting the forests of Eastern Ghats of Andhra Pradesh, India (R.R. Venkata Raju)[2]. Further the investigations on the antitumor property of the selected plant is not reported and therefore the present study was undertaken to evaluate the anti-tumor properties for leaf extracts.

MATERIALS AND METHODS

Collection and authentication of plant material:

Plant material (leaves) was collected during April 2009 and June 2009. The voucher specimens have been deposited at Sri Krishnadevaraya University (SKU), Anantapur. Identification was confirmed with the help of local flora and comparison with authenticated specimens housed at SKU, Anantapur, and MH (Madras) Herbarium, Coimbatore and CAL (Central National Herbarium, Calcutta).

Preparation of extracts:

Extracts of the plant material were prepared by successive solvent extraction method as described below.

The shade dried powdered material of the leaves of *Rhynchosia beddomi* was refluxed successively with the solvents like Petroleum ether (40°-60° E- Merck Mumbai, India), Ethyl acetate (E-Merck Mumbai, India) and Ethanol (E- Merck Mumbai, India) in a Soxhlet extractor for 48 hrs in batches of 350g each. Every time, before extracting with the next solvent the marc was dried. After extraction with ethanol lastly, marc was kept in closed jar in distilled water for 48 hrs with occasional shaking, the filter obtained by pressing the marc with tincture press is dried in hot water bath. All the extracts obtained were concentrated in vacuum using rotary flash evaporator (Buchi-Flawil, Switzerland). The solvents were removed completely over the hot water bath and finally desiccator dried. The extracts so obtained from each of the solvents were labeled, weighed and the yield was calculated in terms of grams percent.

Animals

Healthy *Albino mice* of either sex, weighing 20-30 grams were procured from the animal house of S J M. College of Pharmacy, Chitradurga, India. The animal house was well ventilated and animals had 12 ± 1hr day and night schedule. The animals were housed in large spacious hygienic cages during

the course of the experimental period and room the temperature was maintained at 25 ± 1°C. The animals were fed with standard rat feed (Hindustan Lever Ltd; Bangalore) and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. SJMCP /IAEC /PhD/ PH.CHEM /05/2009-10).

Tumor cells and Inoculation:

Ehrlich ascites carcinoma (EAC) cells were obtained through the courtesy of Amla Cancer Research Centre, Thrissur, Kerala state, India. These cells were maintained in Swiss albino mice by fortnightly intraperitoneal inoculation of 1x10⁶ cells/mouse i.e., the viable EAC cells were counted (Trypan blue indicator) under microscope and were adjusted at 1x10⁶cell/ml.

Preparation of test samples:

Suspensions of the crude extracts *viz*; petroleum ether and ethyl acetate extracts were prepared in 0.5 % Tween 80 so as to obtain the dosage forms in the concentration of 100mg and 200mg/ml. The suspensions so obtained were administered orally to the animals with the help of intra-gastric catheter at the doses of 100mg and 200 mg/kg of body weight for extracts.

Preparation of Standard drug formulation

The standard drug 5-Fluorouracil 20mg/kg body weight was suspended in 0.5% Tween 80 and administered orally to the animals with the help of intra-gastric catheter.

Procedure for Anti tumor testing

The Swiss albino mice were divided into seven groups of eight animals each. All animals were inoculated with 1x10⁶ cells/mouse on the zero days. A day of incubation was allowed for multiplication of the cells.

Fourteen doses of the ethyl acetate, ethyl alcohol extract and 5-Fluorouracil were given orally to the animals with the help of intra-gastric catheter respectively from the first day up to the 14th day with 24 h intervals.

- Group 1 Vehicle control alone
- Group 2 Vehicle control + EAC Cells
- Group 3 Vehicle control + 5 Fluorouracil
- Group 4 Vehicle control + Ethyl acetate extract 100mg/kg body weight
- Group 5 Vehicle control + Ethyl acetate extract 200mg/kg body weight
- Group 6 Vehicle control + Alcohol extract 100mg/kg body weight
- Group 7 Vehicle control + Alcohol extract 200mg/kg body weight

Food and water was withheld 18 h before scarifying the animals. On 15th day, half of the animals (n=4) in each group were anaesthetized slightly with anesthetic ether and blood was collected from retro-orbital puncture and kept at 37°C for 30 min and the remaining animals were continued for survival time. Immediately after collecting the blood samples, the mice were killed by cervical dislocation. The blood so collected was immediately used for the estimation of hemoglobin (Hb) content, red blood cell count (RBC) and white blood cell (WBC). The mean survival time (MST), percentage increase in life span (%ILS) and percent increase in the body weight were calculated out from the rest of the animals^[6]. Antitumor activities of the extracts were measured in EAC induced animals with respect to the following parameters:

Tumour volume The mice were dissected and the ascetic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min.

Tumour cell count The ascetic fluid was taken in a WBC pipette and diluted 100 times with distilled water. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the

numbers of cells in the 64 small squares were counted.

Viable / non-viable tumour cell count The cells were then stained with Trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. Count the number the number of viable and nonviable cells under research microscope^[7].

Percentage increase life span (% ILS) The effect of Extracts on tumour growth was monitored by recording the mortality on daily basis for a period of 6 weeks and percentage increase in life span^[7] (%ILS) was calculated by using the formula;

$$\%ILS = \frac{\text{Mean survival treated group} - \text{Mean survival of control group}}{\text{Mean survival of control group}} \times 100$$

$$\text{Mean survival} = \frac{\text{Day of 1st death} + \text{Day of last death}}{2}$$

Body weight

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period^[8].

$$\text{Increase in Body weight\%} = \frac{\text{Wt. of each animal on 14th day} - \text{Wt. Of each animal on 0 day}}{\text{Wt. Of each animal on 0 day}} \times 100$$

Haematological parameters

At the end of the experimental period, all the mice were sacrificed after 18h of fasting by decapitation. Blood samples collected from retro-orbital puncture were used for the estimation of Haemoglobin (Hb) content, red blood cells (RBC)^[9] count and white blood cell (WBC)^[10] cell counts. WBC differential count was carried out from Leishman stained blood smears^[11].

Estimation of Haemoglobin

0.1ml of heparinised blood was taken in Sahli's hemoglobinometer and diluted with 0.1 NH₄Cl until the colour matched with standard. The

reading was then taken from the graduated cylinder and expressed as mg/100ml of blood.

Estimation of Erythrocyte (RBC) count

For the erythrocyte (RBC) counts, blood samples were diluted 1:200 with the Rees-Ecker diluting fluid containing Sodium citrate (3.8g), HCHO (0.2ml) and Brillint CresylBlue, volume made up to 100ml with sterile distilled water.

Blood samples were drawn by the fine tips (capacity 20 μ l) attached to an auto adjustable micropipette and transferred to the micro centrifuge tubes containing the dilution fluid. The tubes were mixed thoroughly and a drop of the resultant suspension was discharged under the cover glass of an improved Neubuer bright field haemocytometer and the corpuscles were allowed to settle. The numbers of erythrocyte in 80 small squares were counted under light microscope. The number of cells in 1 μ l of undiluted blood was calculated following the standard formula.

Erythrocyte count = $N \times 1 / 0.02 \times 200$.

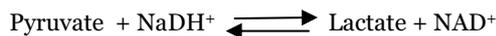
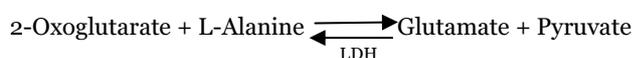
$N \times 10,000$. Where N = no. of cells in 80 small squares i.e. 0.02 μ l

Marker Enzymes and Biochemical Estimations

Marker enzymes such as alanine amino transferase (ALAT), aspartate amino transferase (ASAT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP) were measured spectrophotometrically in serum and liver samples using commercially available Ecoline diagnostic kits (Merck, India) [12].

Assay of Alanine Amino Transferase (ALAT or SGPT)

Alanine amino transferase in serum and liver homogenate was assayed by using Ecoline diagnostic kit. ALAT catalyzes by the following reactions.



Where LDH is lactate dehydrogenase. The rate of NADH consumption was measured photometrically at 340 nm and is directly proportional to the ALAT activity in the sample. Alanine amino transferase level in serum and liver tissue homogenate was expressed as U/L.

Procedure

Reagent 1 and 2 of Ecoline diagnostic kit for ALAT were mixed at the ratio of 4:1 and the temperature was maintained always at 30 °C. To 50 μ l of the sample, 0.5 ml of the reaction solution was added and mixed. After 1 min, the decrease in absorbance was measured every minute for 3 min at 340 nm by using the following equation.

$$\text{Enzyme activity [U/I]} = (\Delta A / \text{min}) \times 2143$$

Where ΔA is the decrease in absorbance per minute

Assay of Aspartate Amino Transferase (AST or SGOT)



Aspartate amino transferase in serum and liver homogenate was assayed by using Ecoline diagnostic kit. ASAT catalyzes by the following reactions.

The rate of NADH consumption was measured photometrically at 340 nm and is directly proportional to the ASAT activity in the sample. Aspartate amino transferase level in serum liver and kidney tissue homogenate was expressed as U/L.

Procedure:

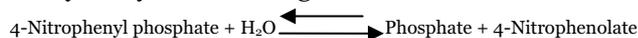
Reagent 1 and 2 were mixed at the ratio of 4:1 and the temperature was maintained always at 30 °C. To 50 μ l of the sample, 0.5 ml of the reaction solution was added and mixed. After 1 min, the decrease in absorbance was measured every minute for 3 min at 340 nm. by using the following formula.

$$\text{Enzyme activity [U/I]} = (\Delta A / \text{min}) \times 2143$$

Where ΔA is the decrease in absorbance per minute

Assay of Alkaline Phosphatase (ALP)

Alkaline phosphatase in serum and liver homogenate was assayed by using Ecoline diagnostic kit. ALP catalyzes by the following reaction:



The rate of increase in 4-nitrophenolate was determined photometrically at 405 nm and is directly proportional to the ALP activity in the sample. Alkaline phosphatase level in serum, liver and kidney tissue homogenate was expressed as U/L.

Procedure

Reagent 1 and 2 were mixed at the ratio of 4:1 and the temperature was maintained always at 30 °C. To 20 μl of the sample, 1 ml of the reaction solution was added and mixed. After 1 min, the increase in absorbance was measured every minute for 3 min at 405 nm. by employing the following equation.

$$\text{Enzyme activity [U/I]} = (\Delta A/\text{min}) \times 2754$$

Where ΔA is the increase in absorbance per minute

Estimation of Total Bilirubin (TB)

The total bilirubin was determined by coupling with diazotized sulfanilic acid after the addition of caffeine, sodium benzoate and sodium acetate. Bilirubin reacts with diazotized sulfanilic acid and forms blue azobilirubin dye in alkaline Fehling solution II. The blue compound was measured spectrophotometrically at 578 nm. The concentration of total bilirubin was expressed as mg/dL.

Procedure

Reagent1 (sulfanilic acid 0.5%, hydrochloric acid 1.5%) and 2 (sodium nitrate) were mixed at the ratio of 4:1 and known as diazo solution was maintained at 30 °C. To 50 μl of the sample, 50 μl of the diazo solution and 250 μl of acceleration solution

(mixture of caffeine 5%, sodium benzoate 7.5% and sodium acetate 12.5%) were added and mixed. The reaction mixture was maintained at room temperature, after 1 hr, 250 μl alkaline Fehling solution II was added and the reaction mixture was incubated at room temperature for 5 min. The absorbance of the sample and standard were measured against the reagent blank after 5 min at 578 nm. by using the following equation.

$$\text{Total Bilirubin (mg/dl)} = (A_{\text{sample}} - A_{\text{blank}}) \times 27.78$$

Where, A_{sample} is absorbance of sample and A_{blank} is absorbance of blank

Assay of Total Protein (TP)

Total protein in serum and liver homogenates were assayed using Ecoline diagnostic kit. Proteins and peptides, in contrast to other nitrogen containing compounds (eg. creatinine, urea and uric acid) produce a violet colored complex with copper ions in an alkaline solution. The so called Biuret reaction is particularly easy to carry out giving reproducible results, which are good in agreement with Kjeldahl method. The absorbance of the colour complex is directly proportional to the protein concentration in sample materials. The absorbance of the sample and standard was measured against the Biuret reagent and the absorbance of the blank against distilled water at 546 nm. Total protein level in serum, liver and kidney tissue homogenates were expressed as g/dL^[13].

Procedure

Reagent 1 and 2 of Ecoline diagnostic kit for assay of total protein were mixed at the ratio of 4:1 and the temperature was maintained always at 37 °C. To 0.10 ml of sample/standard, 1.00 ml of the reagent was added, mixed well and incubated for 5 min, and the absorbance of sample and standard were measured against reagent blank within 60 min. The following formula was used for the measurement.

Total Protein (g/dl) = $(A_{\text{sample}} - A_{\text{blank}}) \times$
Concentration of standard.

Where, A_{sample} is absorbance of sample and A_{blank} is absorbance of blank

Short term toxicity studies

For the determination of short term toxicity, healthy Swiss albino mice were treated intraperitoneally with the single doses of test compounds. The mice which received 100mg and 200mg/kg body weight of both ethyl acetate and alcoholic extracts did not showed any toxic effects. The symptoms such as inactiveness in general behavior, slow movement, loss of appetite, hypothermia, erected hairs etc were absent. The weights of the vital organs (liver, kidney, brain and spleen) were also not affected by these test compounds. Hematological parameters like Hb, RBC and WBC, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were also not unaltered.

RESULTS

Among the two crude extracts of the leaves of *Rhynchosia beddomei* ethyl acetate extract 200 was found to be more potent against the EAC induced

tumor cell growth which can be indicated by the increase in mean survival time, increase in lifespan and decrease in body weight 31.00 ± 0.41 , 61.81 ± 0.41 and 11.42 ± 0.78 respectively. When compared to EAC control 22.00 ± 0.41 mean survival time and 27.52 ± 1.29 increase in body weight. Animals treated with known standard drug 5-fluorouracil showed maximum increase in mean survival time 36.00 ± 0.41 , increase in lifespan up to 63.63 ± 0.45 and decreased body weight by 9.12 ± 0.64 whereas, the result of animals treated with alcoholic extract 200 is 26.25 ± 0.48 , 13.68 ± 0.14 , 17.15 ± 1.49 respectively as shown in Table-1 Fig-2.

Fig-1: A- EAC treated animal, B- Normal control



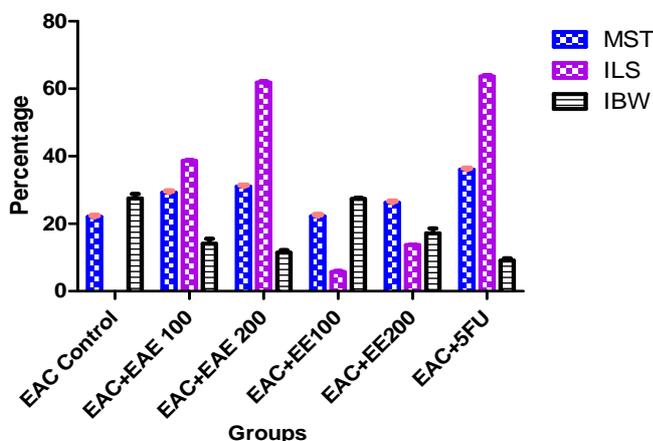
Table 1: Effect of Extracts of leaves of *Rhynchosia beddomei* treatment on the Survival Time and Body Weight of the tumor bearing mice.

Group No.	Mean survival time (MST) in days	Increase in life span ILS (%)	Increase in body weight IBW (%)
Normal control	--	--	--
EAC control (1x10 ⁶ cells)	22.00±0.41	--	27.52±1.29
EAC + Ethyl acetate extract 100	29.25±0.48*	38.64±0.23*	14.1 ± 1.45*
EAC + Ethyl acetate extract 200	31.00±0.41**	61.81±0.41**	11.42±0.78**
EAC + Ethanol extract 100	22.25±0.48	5.68±0.32	27.38±0.32
EAC + Ethanol extract 200	26.25±0.48	13.68±0.14	17.15±1.49
EAC + 5FU(20mg/kg b w)	36.00±0.41**	63.63±0.45**	09.12±0.64**

Values are mean S.E.M. where n=6

*p<0.001 statistically significant when compared with EAC control group.

Fig-2: Effect of Extracts of leaves of *Rhynchosia beddomei* treatment on the Survival Time, Increase in life span and Increase in body weight of the tumor bearing mice.



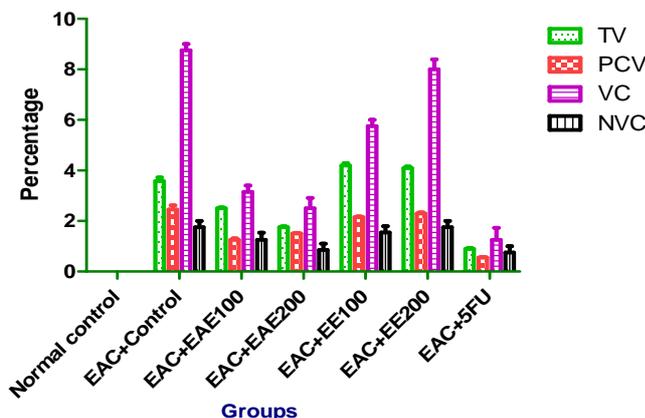
The tumor cell volume, packed cell volume and viable cell count showed 3.58 ± 0.14 , 2.45 ± 0.17 , 8.75 ± 0.25 significant increase in EAC bearing mice control. Administration of ethyl acetate extract 200 showed a significant reversal these parameters 1.75 ± 0.04 , 1.05 ± 0.03 , 2.50 ± 0.41 towards the normal values. Significant reversal of these effects was also observed in the standard 5-fluorouracil treatment 0.90 ± 0.04 , 0.55 ± 0.03 , and 1.25 ± 0.48 . The treatments are shown in (Table No. 2 and Fig 2). It seems that ethyl acetate extract 200 showed significant protection against EAC tumor cells by decreasing the body weight, increase in the lifespan and increase in mean survival time. The graphical representation and the values are shown in Table-2, Fig-3.

Table-2: Effect of extracts leaves of *Rhynchosia beddomei* treatment on the tumor growth

Particulars Groups	Tumor volume (ml)	Packed cell volume (ml)	Viable cells	Non-viable cells
Normal control				
EAC control (1×10^6 cells)	3.58 ± 0.14	2.45 ± 0.17	8.75 ± 0.25	1.75 ± 0.25
EAC + Ethyl acetate 100	2.50 ± 0.04	$1.25 \pm 0.06^*$	$3.15 \pm 0.25^{**}$	$1.25 \pm 0.29^*$
EAC + Ethyl acetate 200	$1.75 \pm 0.04^{**}$	$1.05 \pm 0.03^{**}$	$2.50 \pm 0.41^{**}$	$0.85 \pm 0.25^{**}$
EAC + Ethanol Extract 100 11100 100100extract100	4.20 ± 0.09	$2.15 \pm 0.03^{**}$	5.75 ± 0.25	$1.55 \pm 0.25^{**}$
EAC + Ethanol Extract 200	4.10 ± 0.05	$2.30 \pm 0.04^{**}$	$8.0 \pm 0.4^{**}$	$1.75 \pm 0.25^{**}$
EAC + 5FU(20mg/kg b.w)	$0.90 \pm 0.04^{**}$	$0.55 \pm 0.03^{**}$	$1.25 \pm 0.48^{**}$	$0.75 \pm 0.25^{**}$

Values are expressed as mean \pm SEM, n=six rats in each group, ** p<0.001 compared to tumor control

Fig-3:- Effect of extracts leaves of *Rhynchosia beddomei* treatment on the tumor growth



The anti-tumor property of the extracts was also assessed by studying the different hematological parameters. Hematological parameters of EAC infected mice with Ethyl acetate 200 were found to be remarkably altered when compared to EAC control mice. Modest changes were observed in total WBC count, hemoglobin percentages and total number of RBCs. Treatment with ethyl acetate extract 200 and 5FU 20mg/kg changed these altered parameters to the normal values. The altered hematological parameters for both crude extracts and the standard are given in Tables-3, and Figs-4.

Table-3: Effect of Extracts of leaves of *Rhynchosia beddomei* on Haematological parameters of EAC bearing mice

Groups	Hb content (g/dl)	Total RBC count 10^6 cells/mm ³	Total WBC count 10^3 cells/mm ³	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
Normal control	13.15±0.10	6.00±0.09	5.32±0.09	73.50±1.71	25.00±1.68	1.50±0.29
EAC control (1x10 ⁶ cells)	09.20±0.11*	3.85±0.14*	9.72±0.13*	24.25±1.31*	74.25±1.55*	1.40±0.29
EAC + EAE100	11.13±0.19**	5.46±0.16	6.25±0.16**	61.75±1.21**	48.15±1.08**	1.48±0.29
EAC + EAE200	12.58±0.16	5.92±0.12	5.80±0.19	69.50±1.53	33.25±2.19**	1.35±0.25
EAC + EE100	9.10±0.19**	3.38±0.11**	8.9±0.15**	43.25±1.15**	53.34±1.18**	1.45±0.25
EAC + EE200	10.12±0.12**	4.20±0.15**	8.17±0.11**	49.25±1.55**	42.50±1.18**	1.52±0.29
EAC+5FU(20mg/kgbw) 5-Fluorouracil standard drug	13.12±0.05	5.85±0.10	5.30±0.09	75.75±0.85	23.00±0.82	1.25±0.25

Values are mean S.E.M. where n=6 *p< 0.001statistically significant when compared with normal group. **p < 0.001statistically significant when compared with EAC control group.

Fig-4:- Effect of Extracts of leaves of *Rhynchosia beddomei* on Haematological parameters of EAC bearing mice

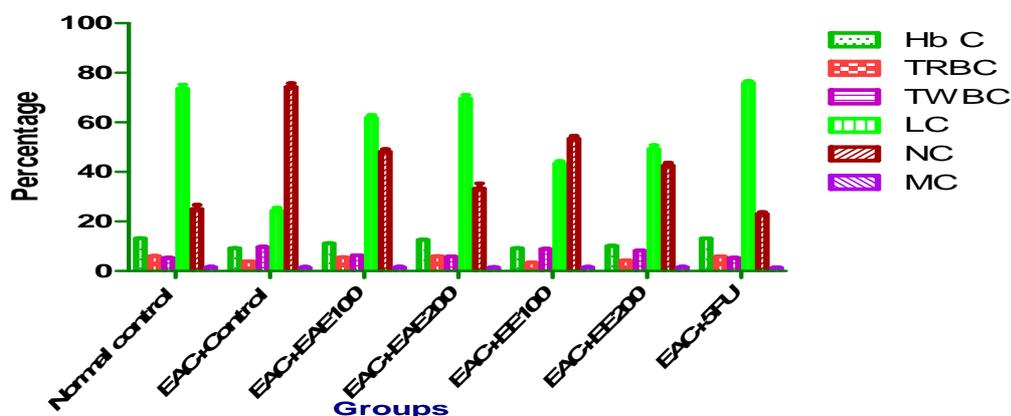


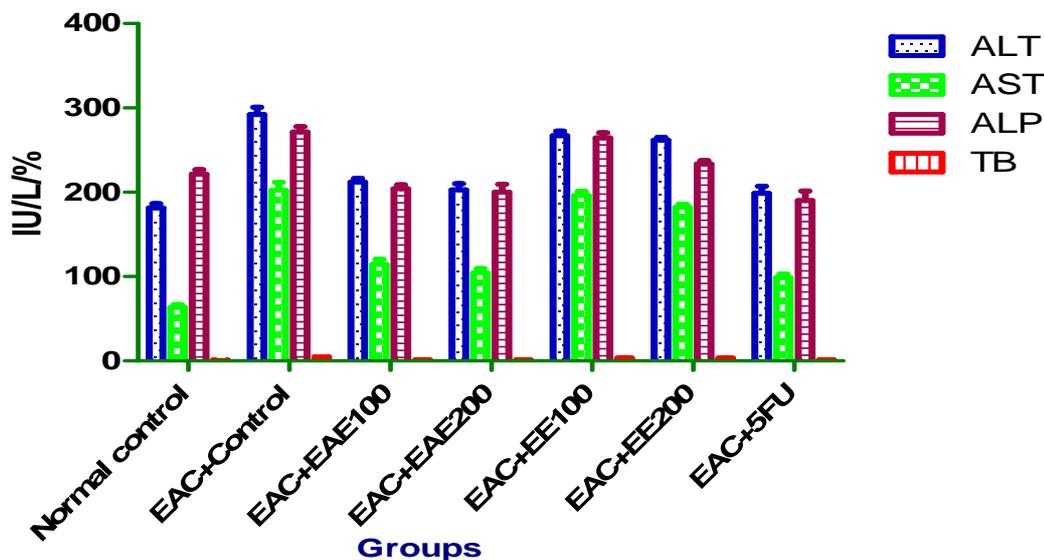
Table-4:Effect of Extracts of leaves of *Rhynchosia beddomei* treatment on marker enzymes of EAC bearing mice.

Groups (n)	ALT (IU/l)	AST (IU/l)	ALP (IU/l)	Total Bilirubin
Normal control	181.5±5.06	63.42±3.44	221.50±5.45	0.56±0.02
EAC control (1x10 ⁶ cells)	292.00±8.75*	202.62±9.30*	271.50±6.30*	4.88±0.10*
EAC + EAE100	212.00±4.45*	114.50±6.08**	204.23±4.54**	1.25±0.15**
EAC + EAE200	202.75±7.47**	104.50±4.95**	200.31±9.21**	1.14±0.17**
EAC + EE100	267.15±5.42	196.00±5.31	264.50±6.14	3.66±0.16
EAC + EE200	261.72±3.43	182.32±3.56	233.5±4.13	3.45±0.19
EAC + 5FU (20mg/kgbw) 5- Fluorouracil standard drug	198.75±8.26**	98.75±4.27**	190.00±11.37**	1.05±0.06**
ANOVA				
f	35.8	90.6	19.1	481.9
df	6,21	6,21	6,21	6,21
p	0.0001	0.0001	0.0001	0.0001

*p values are expressed as mean ± SEM when n=6 rats in each group.

*P < 0.001 compared to normal, **p < 0.001 compared to tumor control

Fig-5: Effect of Extracts of leaves of *Rhynchosia beddomei* treatment on marker enzymes of EAC bearing mice.



Observation were also made for the presence the enzymes such Aspartate amino transaminase (AST), Alanine amino transaminase (ALT), alkaline phosphatase and total bilirubin content in the plasma as these enzymes to leak in to the blood due to liver cell injury. It was also reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the vital organs, especially the liver. During liver injury liver produces a large amount of these enzymes and were secreted to the circulation. Alanine amino transaminase (ALT) is a cytosol enzyme more abundant in liver cells than any other cells.

DISCUSSION

The present experiment was evaluated to investigate the anti-tumor activity of the Ethyl acetate and alcohol extract of *Rhynchosia beddomei* in EAC tumor infected mice. The treatment of Ethyl acetate extract mice at the doses of 200mg/kg and 5-fluorouracil 25mg/kg pronouncedly inhibited the tumor volume, packed cell volume, total cell count

and also regained the hematological parameters near to normal condition.

The best parameters for gauging the effectiveness of any anticancer agents are the prolongation of life span of experimental animals [7, 8]. The reduction of tumor volume, viable total cell count finally reduces the burden and amplified the life span of EAC bearing mice. A regular rapid increase in ascetic fluid tumor volume was observed in EAC control tumor bearing mice. The tumor cells directly draw the nutrition from ascetic fluid; it does mean the ascetic fluid continuously supply the nutritional requirement to tumor cells [14]. Treatment with ethyl acetate extract 200 suppressed the tumor volume, viable cell count and enhanced the life span of the tumor bearing mice [15]. It can be concluded that the ethyl acetate extract by reducing the nutritional fluid volume and inhibiting the tumor growth increases the life span of EAC-bearing mice.

The major problems in the cancer chemotherapy are myelosuppression and anemia [16, 17]. Mainly the reduction of RBC and Hb% in tumor bearing mice lead to the formation anemic condition and this may occur either due iron deficiency or due

to hemolytic or myelopathic conditions^[18]. The treatment with *Rhynchosia beddomei* lignans restored the hemoglobin (Hb) count, RBC and WBC count more or less to normal levels. These finding clearly indicates that leaf extracts of *Rhynchosia beddomei* posses effective protective properties on the hemopoietic system. Over all effects of ethyl acetate and to the some extent alcohol extract on EAC tumor bearing mice might be due to Phytochemical constituents present in it. Phytochemical investigations clearly indicate the presence flavonoids, flavonals and flavones in the ethyl acetate extract. This can be confirmed by the work of Kandaswamy *et al* on flavonoids in edible fruits and vegetables^[4].

There is a much lower risk of colon, prostate and breast cancers in Asians, who consume more vegetables, fruits and tea than populations in the Western hemisphere as the flavonoid components mediate the protective effects of diets rich in these foodstuffs by acting as natural anti-oxidants, chemo-preventive and anticancer agents. *In vitro* work on flavonoids has concentrated on the direct and indirect actions of flavonoids on tumor cells, and found a variety of anticancer effects such as cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and of tumor invasive behavior. Furthermore, some studies have reported the impairment of *in vivo* angiogenesis by dietary flavonoids. Experimental animal studies indicate that certain dietary flavonoids possess antitumor activity. The hydroxylation pattern of the B ring of the flavones and flavonols, such as luteolin and quercetin, seems to critically influence their activities, especially the inhibition of protein kinase activity and anti-proliferation. The different mechanisms underlying the potential anticancer action of plant flavonoids await further elucidation. Certain dietary flavonols and flavones targeting cell surface signal transduction enzymes, such as protein

tyrosine and focal adhesion kinases, and the processes of angiogenesis appear to be promising as anticancer agents. Further *in vivo* studies of these bioactive constituents are deemed necessary in order to develop flavonoid-based anticancer strategies. In view of the increasing interest in the association between dietary flavonoids and cancer initiation and progression, this important field is likely to witness expanded effort and to attract and stimulate further vigorous investigations ^[5].

CONCLUSION

The leaf extracts of *Rhynchosiae bedomei* exhibited antitumor activities against Ehrlich Ascites Carcinoma in Swiss albino mice. Animals induced with EAC cell lines (at a dose of 2×10^6 cells) and then after 24hrs, treatment with ethyl acetate and alcohol extract at a dose of 100mg and 200mg/kg, for a period of 14 days. Later the blood samples were collected and animals were sacrificed for studying of antitumor activity. The decrement of tumor volume, packed cell volume and viable cell count were observed in both ethyl acetate and alcohol extract when compared with fluorouracil treated and EAC control tumor bearing mice. Treatment with ethyl acetate 200 showed significant increase in the survival ime and normal peritoneal cell count. Hematological parameters, PCV which were altered in EAC treated animals, were restored considerably in ethyl acetate treated animals.

By observing the results of the all the parameters, it clearly indicates that the overall effects of plant *Rhynchosia beddomei* is mainly due the horde flavonoid it contains. The inhibitory property of the extracts of *Rhynchosia beddomei* on EAC cell lines can be considered further studies in the treatment of cancer.

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