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ANTI – TUMOUR EFFECT OF BERBERIS ASIATICA ROXB. EX. DC. ON DALTON'S LYMPHOMA ASCITE

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ABSTRACT: *Berberis asiatica Roxb. Ex. Dc., non Griff. Belongs to the family berberidaceae commonly occurring in the dry outer Himalaya, Assam etc. Roots along with stem bark s a reputed drug in Ayurvedic medicine contain several alkaloids. 50% Ethaolnic extract of roots reported to posses anti-cancer activity. The present study examines the antitumour effect of ethanolic root extract (BRE) against Dalton's lymphoma ascites tumour cells and solid tumour in swiss albino mice, A significant enhancement of mean survival time of BRE treated tumour bearing mice was found. Oral administration of BRE reduced the solid tumour induced by DLE and restored the altered haematological parameters to normal.*

INTRODUCTION

Berberis asiatica Roxb.ex DC., non Griff. (Family Berberidaceae) is a pretty ever green thorn shrub 1.8 to 2.4m in height with light brown rough bark and oblong-ovoid edible berries, commonly occurring on the dry outer Himalaya from 600 – 2550m from kumaon east wards and in Assam, Madhya Pradesh, Mount Abu also. (1,2) It is commonly known in vern acular. As *Kilmora or kingora.*

Roots along with stem bark form a repute drug in Ayurvedic medicine. It contains several alkaloids viz. Berberine, Palmitine, Jatrorrhizine, Columbamine, Tetrahydra palmitine, berbamine, Oxyberberine and oxya canthine. (3,4,5) EtoH (50%) extract of roots re reported to possess spasmolytic and anticancer activity.(5,6).

Fruits or berries are given as a mild laxative to children. Stems are recommended in rheumatism. The root's action is as powerful as quinine. Decoction made from the root bring down fever. The dried extract of the root known as "Rasault" or "Ras" is a highly esteemed drug in indigenous medicine. It is bitter, tonic cholaguge used as a purgative for children as a blood – purifier antipyretic antiseptic and for external application in conjunctivitis. It has also been recommended for gastric and duodenal ulcers ad for haemorrhoids both localloy and internally (1,6,7). In Unani system it is used in leprosy. A yellow dye obtained from the roots and stems is of great value in tanning and for colouring leather. (8)

MATERIALS AND METHODS

Plants materials

The fresh roots of B. asiatica Roxb. Ex. Dc., of 15-20 yrs old tree were collected from the village Manjoor 25 km away from Ooty, the Nilgiris, Tamil Nadu and identified at the Department of Pharmacognosy, JSS College of Pharmacy, Ooty with the help of herbarium sheet.

Tumour cells

Dalton's lymphoma ascites tumor cells (DLA) was obtained from Amala cancer research centre, Amala Nagar, Trissur, Kerala.

Chemicals

All the chemicals used in the present stud were of analytical reagent quality.

Animals

Swiss albino mice weighing 17 to 25 gm supplied from our animal house, J.S.S College of pharmacy, Ooty, were housed in well ventilated cages and fed with normal mouse pellet feed (Lipton India) and water libitum were used for the study.

Extraction and preparation of the test sample.

The fresh samples of roots were collected, dried, ground and soaked in 50% aqueous ethnol for a week at room temperature (15-25^{o}C) with occasional stirring. This procedure was repeated twice. The pooled extracts were concentrated, evaporated to dryness under reduced pressure. The yellow solid residue was stored at 4^{o}C in a closed container and designated as BRE (*Berberis asiatica* Root Extract) for our experiments. The extract was suspended in 1% gum acacia suitable dilutions were made and subjected to the various experimental studies.

Effect of BRE on DLA tumour model

Three groups (a,b,c) of animals were transplanted with one million cells of DLA intraperitoneally. a,b,c group received BRE 0.1mg, 0.2mg and 0.4 mg/g body weight respectively once / day orally for 10 days. According to the schedule of drug administration the mice from all the three groups were further divided into 4 groups (9,10,11).

Groups 1 animals were treated 24 hours after tumour transplantation once daily for 10 days.

Group 2 animals were treated once daily form the eleventh day of tumour transplantation for 10 days.

Group 3 animals were treated once daily for 10 days prior to tumour transplantation.

Group 4 animals were served as control treated with gum acacia (1%).

All the mice were weighted on the day of tumour transplantation and at weekly intervals. Animal survival was monitered up to 40 days mean survival times (MST) were noted. Survival times of treated groups (1,2,3) were compared with those control group (4) and the % increase in life span (T/C%) was calculated (12,13) by the formula:

% Increase in life span = T/C%

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

Effect of BRE on Haematological Parameters

In order to detect the influence of BRE on the hematological status of DLA bearing mice. The comparison was made amongst the above mentioned four groups of mice on the 14th day after transplantation with normal mice

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Blood was drawn from each mouse in the conventional way an white blood cells count (WBC), red blood cells count (RBC), differential count and haemoglobin (Hb) percentage were estimated. (15)

Acute oral toxicity

The dose response survival studies were performed after oral administration of the BRE in 1% gum acacia in graded doses, mortality was monitored for a period of 14 days. The LD 50 values were calculated (14)

Effect of BRE on solid Tumour model

Three groups (I,II,III) of animals were transplanted with one million cells each of DLA subcutaneously into the right hind limb for the solid tumour development. Group I, II and III were received BRE o.1 mg and 0.4 mg/g body weight one daily orally for 10 days respectively. The three groups of he animals were further subdivided into 4 groups according to the schedule of drug administration and designation as group A,B,C and D (9,10,11)

Group A animals were treated 24 hours after tumour transplantation once daily for 10 days.

Group B animals were treated once daily from the eleventh day of tumour transplantation for 10 days.

Group D animals were served as control treated with gum acacia (1%).

Solid tumours were measured form day 7^{th}, 14^{th} , 21^{st}, 28^{th} of transplantation the volume was calculated using the formula $V= /3\pi r_1^2$, r_2^2. Where r_1^2 & r_2^2 are radii of tumours. (10).

RESULTS

Acute oral Toxity

The LD_{50} for BRE was found to be 1738 mg/kg body weight (Table No.1) At dose of 500, 1000, 1500 mg/kg. no mortality was observed when the dose was increased the mortality was higher. At higher doses toxic systems like drowsiness, toxic systems like drowsiness, lethargy were observed. The animals which could survive the lethal effects upto 3 days were able to survive and continue even upto 14 days.

Survival time

The effect of BRE on the survival of tumour bearing mice showed (Table No 2) the mean survival time for the control group to be 25 days while it was 36 days (T/C % = 144) for the group treated for 10 days wit BRE (200 mg/kg oral /day) 10 days after the tumour transplantation. The MST of the group treated wit the higher dose (400 mg/kg oral/day) was formed to be 36 days only. The treated for 10 days sowed no 60 days survivors.

Haematological Studies

Haematological parameters (Table 4), of tumour bearing mice on day 14 were found to be significantly altered from the normal group. The total WBC count was found to be increased In a differential count of WAC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval, BRE (400 mg/kg/day oral) treatment for 10 days a) after 24hrs tumour transplantation b) 10 days after tumour transplantation could restore those altered parameters near to normal.

Solid Tumour

The effect of BRE on solid tumours is shown in Table 5. Treatment with BRE reduced the solid tumour volume considerably. The maximum solid tumour volume reduction was sown in treatment with 200 mg/kg body et/day/oral for 10 days 24 hrs after tumour transplantation groups.

For example tumour volume of untreated mice was 0.19 ml on7th day, 1.9 ml and on the 14^{th} day, 3.3 ml on the 28^{th} day while for t treated group the tumour volume was nil on the 7^{th} as well on the 14^{th} day, 0.7 ml on the 21^{st} day and 2.6 ml on the 28^{th} day. The post treatment with 200 mg/ kg for 10 days 24 hr after tumour transplantation sowed a considerable reduction in tumour volume.

DISCUSSION AND CONCLUSION

Anti – cancer activity of Berberis asiatica as been established (5,6). Present studies demonstrate the BRE is also effective against DLA which regressed after the treatment mean survival time of untreated tumour transplantation animals is about 25 days with gradual increase in tumour volume till the death of the animals. The volume of ascites fluid increases more rapidly in the ascites tumour during tumour growth. Ascites fluid is the direct nutritional source to the tumour cells and the faster increase in ascites fluid with tumour growth (16). Treatment with BRE has shown the increase in the MST to 36 days and decrease in tumour volume which is comparable with average body weight change. This significant enhancement of MST was found which was dose-dependent. The antitumour effect of BRE was ore pronounced but failed to show any curative effect (no 60 day survivors)

Analysis of the haematological parameters showed a minimum toxic effect in the mice which were cured by BRE treatment. 14 days after tumour trans plantation, BRE treated groups were able to reverse the change in the haematological parameters.

Effect of BRE on solid tumour reduction studies exhibited a significant tumour reducing property which was compared on 28^{th} day wit normal.

All these data point to the possibility of developing *Berberies asciatica* root extract as a novel potential agent in the area of cancer chemotherapy.

Earlier reports indicated the presence of several alkaloids (5,6) further studies on the active principles of BRE to be carried out to confirm the anticancer activity.

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Table No 1
Acute Oral Toxicity

Group	Dose mg/kg	Log Dose	Dead/Total	% Dead	Corrected	Probit
1	1500	3.18	0/10	0	2.5*	3.04
2	1600	3.20	1/10	10	20	3.72

3	1700	3.23	4/10	40	40	4.75
4	1800	3.26	7/10	70	70	5.25
5	1900	3.28	9/10	90	90	6.28
6	2000	3.30	10/10	100	97.5*	6.96

N= number of animals (10 in each group)
*Corrected%:
For the 0% Mortality = 100 (0.25/n)
For the 100% Mortality = 100 (n-0.25/n)
LD50 of the BRE: 178 mg/kg body weight

Table No 2
Effect of BRE on survival time

Group & treatment	MST	% Increase in Life Span
1 a	31.8 ± 1.2 ***	128
b	32.3 ± 1.4 ***	130
c	3.4 ± 1.4 ***	137
2 a	33.7 ± 1.4 ***	135
b	35.5 ± 1.36 ***	143
c	35.8 ± 1.7***	144
3 a	24.3 ± 0.8*	98
b	24.0 ± 0.6*	98
c	24.6 ± 1.22*	88
4.1% Gum Acacia	2.8 ± 0.65	

a,b,c received 100, 200, 400 mg/kg body weight

Group 1: Received the treatment 24 hrs after Tumour transplantation.
Group 2: Received the treatment 11th day after tumour transplantation.
Group 3: Received the treatment 10 days prior to tumour transplantation.

Values marked with *,**,*** indicated the significance (P<0.5, p<0.01, p<0.001, respectively).

Values are mean ± S.E.M. of six animals in a group.

Table No 3
Effect of BRE on Body Weight

Group & treatment	Body Weight	% Decrease in Body Wt
1 a	4.7 ± 0.1 ***	31
b	3.3 ± 0.2 ***	52
c	3.35 ± 0.15 ***	52
2 a	5.6 ± 0.21 **	19
b	4.7 ± 0.31 **	31

c	4.7 ± 0.1***	31
3 a	6.5 ± 0.3*	5
b	6.0 ± 0.2*	13
c	6.0± 0.3*	13
4.1% Gum Acacia	6.9 ± 0.4	

a,b,c received 100, 200, 400 mg/kg body weight

Group 1: Received the treatment 24 hrs after Tumour transplantation.
Group 2: Received the treatment 11th day after tumour transplantation.
Group 3: Received the treatment 10 days prior to tumour transplantation.

Values marked with *,**,*** indicated the significance (P<0.5, p<0.01, p<0.001, respectively).

Values are mean ± S.E.M. of six animals in a group.

Table No 4
Effect of BRE on Haematological parameters

Group & treatment	Total WBC cells /ml x 106	Total RBC cells /ml x 106	Hb (g%)	Differential Count%		
				Lymphocyte	Neutrophil	Monocyte
1 a	19.5 ± 0.8***	8.7 ± 2.1*	14.3 ± 0.2*	43 ± 1.5***	53.5 ± 1.2***	3.2 ± 0.2*
b	17.3 ± 1.4***	8.1 ± 1.8*	13.3 ± 0.3*	58.1 ± 1.6***	40 ± 1.4 ***	2.1 ± 0.12*
c	16.0 ± 0.3***	8.7 ± 1*	14.1 ± 0.1*	58 ± 1.3***	40.2 ± 6.8 ***	.3 ± 0.25*
2 a	18.6 ± 1.9 **	9.1 ± 0.8*	14.1 ± 0.2*	46 ± 1***	52.1 ± 1***	2.5 ± 0.2*
b	16.4 ± 1.6 ***	8.5 ± 0.7*	14.3 ± 0.3*	56± 1.2***	43.3 ± 1.2***	3.1 ± 0.15*
c	16.0 ± 1.3 ***	8.3 ± 1.1*	13.8 ± 0.5*	55 ± 1.9***	42.7 ±1.7***	3.4 ± 0.22*
3 a	25.2 ± 1.1 ***	9.4 ± 05*	14.1 ± 0.4*	35.1 ±2*	62.3 ± 1.6*	2.7 ±0.4*
b	24.3 ± 1.9*	8.3 ± 1.2*	13.8 ± 0.35*	37.4 ± 1.3*	61.4 ± 0.9*	2 ± 0.21*
c	24.3 ± 1.2*	8.7 ± 1*	13.9 ± 0.32*	37.3 ± 1.4*	61 ± 1.4*	2± 0.45*
4.1% Gum	25.3 ± 0.9***	8.81 ±1*	13.8 ± 0.4*	34 ± 1.1***	64.2 ± 1.7***	2.3 ± 0.4

Acacia						
Normal	12.7 ± 1.4	8.9 ± 1	13.6 ± 0.9	65.8 ± 1.4	33.1 ± 1.1	

a,b,c received 100, 200, 400 mg/kg body weight

Group 1: Received the treatment 24 hrs after Tumour transplantation.
Group 2: Received the treatment 10 day after Tumour transplantation.
Group 3: Received the treatment 10 days prior to Tumour transplantation.

Values marked with *,**,*** indicated the significance (P<0.5, p<0.01, p<0.001, respectively).

Values are mean ± S.E.M. of six animals in a group.

Table No 5
Effect of BRE on Tumour Model

Group & treatment	Tumour Volume in Cubic Centimeter			
	7^{th} Day	14^{th} Day	21^{st} Day	28^{th} Day
A I	----	----	0.94 ± 0.09 ***	3.08 ± 0.29 ***
II	----	----	0.7 ± 0.8***	2.56 ± 0.165 ***
III	----	----	0.7 ± 0.8***	3.08 ± 0.12***
B I	Not determined	1.5 ± 1.4 **	1.98 ± 0.073**	4.88 ± 0.16 ***
II	Not determined	1.46 ± 0.13 **	1.84 ± 0.04 **	4.42 ± 0.39***
III	Not determined	1.52 ± 0.13 **	0.08 ± 2.02 **	4.46 ± 0.17 ***
C I	0.12 ± 0.01 ***	1.82 ± 0.22*	3.3 ± 0.24*	6.78 ± 0.21*
II	----	1.8 ± 0.12*	3.2 ± 0.21*	6.2 ± 0.15*
III	----	1.9 ± 0.12*	3.2 ± 0.19*	6.6 ± 0.25*
D1% Gum Acacia	0.19 ± 0.02	1.90 ± 0.29	3.30 ± 0.35	6.70 ± 0.26

I,II,III received 100, 200, 400 mg/kg body weight

Group A: Received the treatment 24 hrs after Tumour transplantation.
Group B: Received the treatment 10 day after Tumour transplantation.
Group C: Received the treatment 10 days prior to Tumour transplantation.

Values are mean ± S.E.M. of six animals in a group.

Group A, B, C were compared with D.

Values marked with *, **, *** indicated the significance (P<0.5, p<0.01, p<0.001, respectively).

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