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A Preliminary Note on the Activity of *Diospyros montana* Bark Extract on *Ehrlich Ascites carcinoma* in Mice

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THE stem bark of *Diospyros montana* Roxb. has been said to be useful in treatment of tumours[1]. Since no systematic investigation to establish its anti-tumour activity has been reported so far, it was decided to study the action of *D. montana* bark extract on *Ehrlich Ascites Carcinoma* (E.A.C.) as the tumour model with Swiss Albino mice as its host. A preliminary attempt has also been made to study the haematological parameters of the mice treated with the crude extract with respect to the control.

Experimental

Materials and methods: Stem bark of *D. montana* was dried and powdered. An extract of the dried powder was prepared with 90% ethanol followed by removal of the solvent in vacuo, whereupon a dark-coloured solid was obtained (C.P.). E.A.C. was routinely maintained in male Swiss A. mice. They were always fed with fixed standard mice diet and water *ad libitum*. Two groups of mice (18-20 g), six of them in each group, were inoculated i.p. with 1.8×10^6 E.A.C. cells (washed and viable) as done previously[2]. Daily treatment of one group (E) with a solution of C.P. in propylene glycol was started 24 hrs after implantation, while the control group (C) was injected with the same volume of the vehicle only (0.05 ml of propylene glycol). LD_{50} value for the C.P. was found to be 800 mg/kg (i.p.) in male Swiss A mice. The experimental group was treated with 200 mg/kg/day of C.P.[3] intraperitoneally for sixteen consecutive days. Another group (A). containing six normal healthy mice (18-20 g) was treated with 200 mg/kg/day of C.P. in propylene glycol in the same way and for the same period. All the groups of mice were observed and weighed regularly. Survival of all the groups were recorded. The growth of tumour was found out as measured weight minus initial weight of each mouse in g[4]. A comparative picture of the growth of tumour in E-group with respect to that in C-group for first twenty days has been shown in Fig. 1. The inhibition of growth was statistically evaluated by Student's t-test. The increase of the life span of E-group of animal over the C-group was calculated as done previously[5] using the formula :

$$\text{Increases in life span} = \left(\frac{\text{Survival time of treated group}}{\text{Survival time of untreated group}} - 1\right) \times 100$$

For haematological study, comparison was made amongst three groups of mice (18-20 g) on the 20th day after tumour transplantation on the E- and C-groups. The first group (N-) comprised of six normal healthy mice, the second group being the mice survived in E-group (five out of six) and the third group consisted of the mice surviving in C-group (four out of six). Blood was taken from the tip of the tail of each mouse and counted for total R.B.C. and total W.B.C. using cell-diluting fluids and hemocytometer[6]. Differential count of W.B.C. was done with Leishman stain. The results have been given in Table 1 and Fig. 2 respectively.

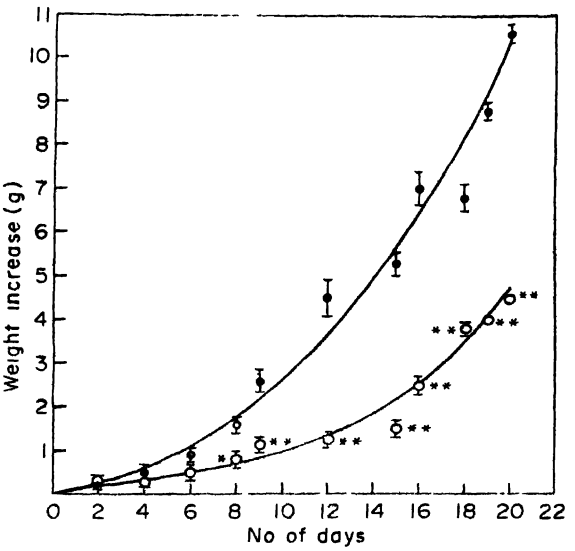


Fig. 1. Effect of *Diospyros montana* bark extract on tumour growth after the inoculum of 1.8×10^6 E.A.C. cells in Swiss mice. Tumour growth, expressed as weight increase in mice above their initial weight, is plotted against the number of days. Each point represents the mean of weight increase of six mice ± S.E.
—○— Experimental ; —●— Control.
*P<0.05, **P<0.001. (Student's t-test).

Table 1—R. B. C. and W. B. C. Counts of Mice Treated with *D. montana* Extract as Compared to Normal Mice

Test Group	R. B. C. Count ($\times 10^6$/cm^3)	W. B. C. Count ($\times 10^6$/cm^3)
Normal Mice (N)	9.35 ± 0.25	9.76 ± 0.29
Control Mice (C)	9.26 ± 0.41†	21.53 ± 0.66*
Experimental Mice(E)	8.59 ± 0.32†	11.02 ± 1.06†

*P<0.001, †P>0.05, as per Student's t-test.

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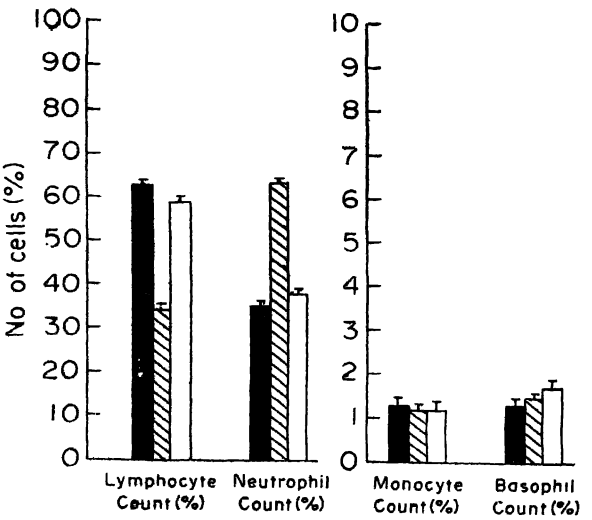


Fig. 2. Differential counts of bloods taken from three groups of mice, six in each.
Group 1 : Normal healthy mice (▒).
Group 2 : Mice inoculated with 1.9×10^6 E.A.C. cells and treated with *Diospyros montana* bark extract (□).
Group 3 : Control mice inoculated with 1.8×10^6 E.A.C. cells (▨).
Vertical bars represent S.E.M.

Results and Discussion

For the mice in A-group there was practically no significant change in weight of the mice even after twenty days. So the result related to A-group has not been incorporated in Fig. 1. Out of six mice in A-group, one died on 45th day and the others were all alive even after sixty days. While the number of mice survived in E-group after thirty days was four out of six, none of the mice in the C-group survived upto thirty days. From Fig. 1 it is evident that on treatment with C. P., a remarkable regression in the growth of E.A.C. in E-group over the C-group is observed throughout the experiment. From Students' t-test it is found that the difference between E-group and C-group becomes significant from the eighth day after transplantation. It is observed that the mean life span of the E-group is 41 ± 3, while that of C-group is 26 ± 2. This indicates an increase of life span of almost 60% for the E-group over the C-group. Table 1 indicates that the mean R.B.C. counts of E- and C-groups do not show any significant departure from that of N-group. However, the W.B.C. count of C-group is found to be increased by 120% over that of N-group while there is no significant difference between the W.B.C. counts of E- and N-groups. Differential count of W.B.C. as shown in Fig. 2 gives a vivid picture of the abnormal haematological pattern of tumour-bearing mice. The drastic increase of neutrophil count in C-group as compared to that of N-group, coupled with the enhancement of total W.B.C. count of the former group, indicates acute neutrophilia of the C-group. In contrast, the lymphocyte and neutrophil counts of E-group do not vary significantly from those of N-group indicating restoration of normal blood picture on treatment with C.P. This corroborates the possibility of the use of *D. montana* bark extract to inhibit the growth of E.A.C. in mice. It will be our next attempt to characterise the active principles in C.P. by carrying out elaborate fractionation followed by extensive bio-assay.

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α-Amyrin Acetate from *Catharanthus rosea* Linn

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CATHARANTHUS rosea Linn (Syn. *Vinca rosea* Linn) has been the subject of numerous investigations for its alkaloidal constituents. The intense interest in the chemistry of the plant is due to the novelty[1] in structural types of alkaloids in the plant and the role of the plant has played in the understanding of the biogenesis[2] of indole alkaloids.

We were interested to examine the neutral constituents of the plant. The petroleum-ether extract of the air dried powdered stem of *Catharanthus rosea* by column chromatography over silica gel furnished a crystalline compound, $C_{32}H_{52}O_2$, M^+ 468, m.p. 224°, $[\alpha]_D^{18} + 77°(C_6H_6)$. The ir spectrum showed characteristic band for acetoxy group at 1730 cm^{-1}. The pmr spectrum (90 MHz, $CDCl_3$) showed signals at 5.2 (m, 1H), 4.5 (m, 1H), 2.01 (S, 3H).

The product and its deacetylated product were compared directly with authentic samples showing the triterpene isolated was α-amyrin acetate.