# Antitumour activity of Biochanin-A against Dalton's Ascitic Lymphoma

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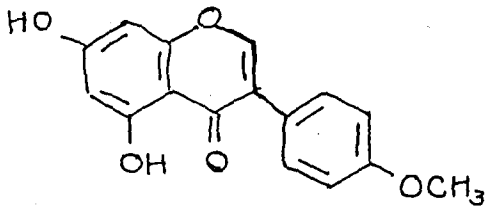
**The antitumour activity of biochanin-A (5,7-dihydroxy-4-methoxy isoflavone) has been evaluated against Dalton's ascitic lymphoma (DAL) in swiss albino mice. A significant enhancement of mean survival time of biochanin-A treated tumour bearing mice was found with respect to control group. Biochanin-A treatment was found to enhance peritoneal cell counts. When these biochanin-A treated animals underwent i.p. inoculation with DAL cells, tumour cell growth was found to be inhibited. After 14 days of inoculation, biochanin-A is able to reverse the changes in the haematological parameters, protein and PCV consequent to tumour inoculation.**

MANY naturally occuring substances were tested for anticancer activity on experimental animals resulting in the present availability of some 30 effective anticancer drugs[1]. So, the present study is focused on the evaluation of antitumour activity of biochanin-A isolated from the flowers of *Dalbergia sissoides*.[2]

Structure of biochanin-A




Biochanin-A isolated, identified and characterised[3] from the flowers of **Dalbergia sissoides** was dissolved in phosphate buffer (pH-7.4) and used in the present study.

Swiss albino mice (20-24 g) were used throughout the study. They were housed in standard microlon boxes and were given standard laboratory diet and water *ad libitum*. Dalton's ascitic lymphoma (DAL) cells were obtained through the courtesy of Cancer Research Institute, Adayar, Madras. DAL cells were maintained by weekly intraperitoneal (i.p.) inoculation of $10^6$ cells/mouse.

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Animals were inoculated with 2-3 x $10^5$ cells/mouse on day O, and treatment with biochanin-A started 24 h after inoculation, at a dose of 10 mg/kg/day i.p. (Group-A). The control group (Group-B) was treated with same volume of 0.9% sodium chloride. All treatments were continued for nine days. Median survival times (MST) for each group, containing 10 mice were noted. The antitumour efficacy of biochanin-A was compared with that of 5-fluorouracil (20 mg/kg/day i.p. for 9 days). MST were noted with reference to control. Survival times of treated groups (T) were compared with those of control groups (C) using the following calculation[4].

$$\text{Increase of life} = T/C\% = \frac{\text{MST of treated group}}{\text{MST of control group}} \times 100$$

Studies of *in vivo* tumour cell growth inhibition with biochanin-A carried out under similar experimental conditions as stated above; using the dose of 10 mg/kg/day for 6 days. Animals were sacrificed on day 7 after inoculation and tumour cells were collected by repeated intraperitoneal wash with 0.9% NaCl, viable tumour cell counts (trypan blue test) were made using a haemocytometer. Total number of viable cells per animal of the treated group was compared with those of the control group.

Three groups of normal mice (n = 5) were used for the study. One group was treated with 10 mg/kg/i.p of

**Table I : Effect of inoculation with Biochanin-A or 5 FU treated DAL**

| Treatment | MST (in days) | Increase in life span T/C % |
|---|---|---|
| Biochanin-A (10 mg/kg i.p) | 31 | 134.70* |
| 5 FU (20 mg/kg i.p) | 40 | 173.90 |
| Saline | 23 | 100.00 |

*P < 0.01
Number of animals used : 10 in each group
Days of drug treatment : 9

**Table 2**
**Effect of Biochanin-A (10 mg/kg i.p) treatment on enhancement of Peritoneal cells in normal mice**

| Experiment | Number of peritoneal cells/ mouse x $10^6$ |
|---|---|
| Control | $6.1 \pm 0.8 \times 10^6$ |
| Treated once | $7.7 \pm 0.6 \times 10^6$ |
| Treated twice on two consecutive days | $8.6^* \pm 0.5 \times 10^6$ |

*P < 0.01
Number of animals used : 5 in each group
Values were expressed as mean ± SE

biochanin-A, while the second group received the same treatment for two consecutive days. The untreated third group was used as control. Peritoneal exudate cells were counted 24 h after treatment for each of the treated groups and compared with those of the untreated group.

In order to detect the influence of biochanin-A on the haematological status of DAL bearing mice, comparison was made amongst three groups (n=5) of mice on the 14th day after inoculation. The three groups comprised (1) tumour bearing mice (2) tumour bearing mice treated with biochanin-A (10 mg/kg/day i.p. for 9 days) and (3) control mice. Blood was drawn from each mouse in the conventional way and the white blood cell count, red blood cell count, haemoglobin, protein and packed cellular volume were determined[5,6]. All the results were analysed by analysis of variance[7].

The effect of biochanin-A on the survival of tumour bearing mice showed MST for the control group to be 23 days, while it was 31 days and 40 days for the groups treated with biochanin-A (10 mg/kg/ day i.p.) and 5 FU (20 mg/kg/day i.p.) respectively.

On day 7 after inoculation, the i.p. DAL cell count (control) was $2.48 \pm 0.34 \times 10^8$ cell/mouse. This value was significant ($P<0.01$) when compared to that of control. The sizes of the DAL cells harvested from treated mice were appreciably smaller with respect to the control cells.

The average number of peritoneal exudate cells per normal mouse was found to be $6.1 \pm 0.8 \times 10^6$. Biochanin-A (10 mg/kg) treatment increased the number of peritoneal cells as shown in Table-2. Single treatment enhanced peritoneal cells to $7.7 \pm 0.6 \times 10^6$ while two consecutive treatments enhanced the number to $8.6 \pm 0.5 \times 10^6$.

Haemotological parameters of (Table 3) tumour bearing mice on day 14 were found to be significantly altered from the normal group. The total WBC count, protein and PCV were found to be increased with a reduction of the haemoglobin content of RBC. In a differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval, biochanin-A (10 mg/kg/day i.p.) treatment could change those altered parameters to near normal.

The reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of the animal[8] and disappearance of WBC from blood[9]. The above results demonstrated the antitumour effect of biochanin-A against DAL in swiss albino mice. A significant enhancement of MST was found. Our observation of inhibition of *in vivo* tumour cell growth after biochanin-A treatment appeared to correlate with the finding of enhancement of survival time by biochanin-A with respect to control. The harvested viable cells after biochanin-A treatment showed morphological changes as revealed by a reduction in the size of the cells. Biochanin-A treatment was found to enhance peritoneal cell counts.

Analysis of the haematological paramters showed minimum toxic effects in the mice which were on biochanin-A treatment. After 14 days of transplantation, biochanin-A treated groups were able to reverse the changes in the haematological parameters consequent to tumour

Table 3: Effect of Biochanin-A (10 mg/kg/day i.p.) on haematological parameters

| | Hb | Total RBC Cells/ml x $10^{10}$ | Total WBC Cells/ml x $10^{6}$ | Protein g% | PCV mm | Differential count | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Lympho Cyte | Neutro phils | Mono-cyte |
| Normal mice | 15.2 ± 0.8 | 1.45 ± 02 | 7.8 ± 0.6 | 8.7 ± 0.7 | 17 ± 0.6 | 68 ± 3 | 30 ± 2 | 2 ± 0 |
| Tumour bearing mice (14 days) | 12.6 ± 0.4 | 1.26 ± 0.4 | 14.2 ± 0.7 | 12.6 ± 0.8 | 24 ± 0.7 | 30 ± 2 | 69 ± 3 | 1 ± 0 |
| Treated tumour bearing mice | 13.1 ± 0.7 | 1.32 ± 0.15 | 10.7* ± 0.4 | 10.4 ± 0.8 | 20 ± 0.8 | 57* ± 3 | 41 ± 2* | 2 ± 0 |

*P < 0.01
Number of animals : 5 in each group
Days of drug treatment - 9
Values were expressed as mean ± SE

inoculation.

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