Effect of *Abrus precatorius* L. on Experimental Tumors

V. V. Subba Reddy and M. Sirsi

*Microbiology and Pharmacology Laboratory, Indian Institute of Science, Bangalore 12, India*

**SUMMARY**

A protein extract isolated from the seeds of *Abrus precatorius* L. is shown to exhibit antitumor activity on Yoshida sarcoma (solid and ascites forms) in rats and a fibrosarcoma in mice. The intraperitoneal route of administration is more effective than subcutaneous injections. The extract has a direct cytotoxic effect on the tumor cells. Vacuolation and disruption of cytoplasm accompanied by karyolysis and chromosomal abnormalities are seen in ascites tumor cells treated with the protein *in vivo*. This is also confirmed by *in vitro* studies. The tumor cells incubated with the extract showed cellular pathology, decreased viable cell counts, and prolongation of survival period of the tumor-transplanted animals.

**INTRODUCTION**

The successful introduction of alkaloids in human cancer therapy has focused attention on plant products as important source materials for obtaining anticancer agents (6). Anticancer screening programs on plant products are being extensively carried out in many laboratories all over the world and have drawn attention to some potentially useful substances.

In our earlier studies, the seed extract of *Abrus precatorius* L. has shown striking effects on mitotic and meiotic chromosomes in the testes of the grasshopper, *Poecilocera picta* (3). Since chemicals which cause chromosomal abnormalities are known to influence the growth of tumors, this extract has now been tested for its effect on some transplanted tumors.

**MATERIALS AND METHODS**

**Tumor Lines**

**Yoshida Ascites Sarcoma (YAS).** Maintenance and tumor progression has been detailed earlier (7). This tumor is carried by an isogeneic substrain of Wistar rats A/IISc and is transferred by i.p. injection of 10⁷ cells once in 4 days for maintenance.

**Yoshida Sarcoma (YS).** Tumor was obtained by s.c. implantation of ascites tumor cells into an allogeneic strain of rats. For maintenance, the tumor was transplanted aseptically every 2 weeks by s.c. trocar injection of pieces (2—4 mm) into the axillary region of rats.

**Mouse Fibrosarcoma (MFS).** This sarcoma, induced by s.c. injection of 6:12-dimethylbenzo-1:2-b:4:5-b'-dithionaphthene in an isogeneic SWR mouse (8), was obtained from Indian Cancer Research Centre, Bombay. This is being maintained in the substrain of mice SWR/IISc by the same technic as with YS.

**General Procedures**

The protein fraction of *A. precatorius* L. seeds (scarlet variety) was prepared as per the method adopted by V. B. Desai and M. Sirsi (personal communication). In brief the procedure was as follows: 25 gm of cotyledons shelled out of commercially obtained seeds were extracted with 250 ml of cold 10% sodium chloride (w/v) solution for 2 days. The supernatant separated at 9000—10,000 rpm was dialyzed for two days and recentrifuged. This supernatant was freeze-dried and stored.

Seed extract solutions were prepared freshly by dissolving the above freeze-dried material in sterile saline such that the required concentrations are contained in 0.2 ml of the solution, i.e., the volume injected s.c. or i.p. each time.

Toxicity and evaluation of antitumor activities of the extract were carried out in healthy rats weighing 120—150 gm and mice weighing 20—25 gm of the respective strains. These animals were provided with water ad libitum and a dry diet composed of cracked wheat 60%, cracked Bengal gram 20%, fish meal 8%, shark liver oil 2%, peanut oil 5%, commercial casein 4%, and common salt 1%. Experimental rats and mice transplanted with YAS, YS, and MFS tumors, as per the technic used for maintenance, were divided into groups of 25 each with a sufficient number of controls which received only saline. Treatment was started the day after transplantation with a single daily dose and continued for 5 consecutive days. With YAS, the effect of a single dose given 24 hours after transplantation was also determined.

Cytomorphologic effects were studied by daily analysis of the aspirated ascites fluid. The first smear was taken 24 hours after the treatment started. Later smears were taken prior to administering the next dose of the extract i.p. Smear analysis was continued until the control hosts died. Regular observations were made on weights and general behavior of all the animals until their death or sacrifice.

To study the cytologic effects of seed extract on developed tumor, rats injected with YAS 5 days earlier were used.
RESULTS

Toxicity Studies

Toxicity was determined for single and multiple doses of varying concentrations by different routes. In rats, 40 μg/kg i.p. as a single dose was lethal, while 25 μg/kg was well tolerated. Subcutaneously, 100 μg/kg was lethal and 75 μg/kg was nontoxic. In multiple doses given consecutively for five days, 7.5 μg/kg and 25 μg/kg were toxic by i.p. and s.c. routes respectively. In mice, 40 μg/kg s.c. as a single dose and 3 μg/kg i.p. for 5 consecutive doses were toxic, while 25 μg/kg s.c. as a single dose and 2 μg/kg i.p. in 5 doses were nontoxic.

In all tumor systems, the effects of well-tolerated doses were determined. For YAS, effect of higher and lower doses by multiple injections i.p. were also determined.

Yoshida Ascites Sarcoma. Doses administered, survival period, and T/C values are shown in Table 1. Five consecutive daily doses of 5 μg/kg i.p. gave the maximum effect, with a survival period of 25 days and a T/C value of 310, as compared to 8 days survival of untreated animals. Lower doses of 0.5 and 2.5 μg/kg were proportionately less effective. Higher doses of 7.5 and 10 μg/kg proved to be toxic. This was revealed by the fact that, though at the cellular level the diminution, disruption, and degeneration of the ascites cells were greater, the enhancement of the survival period of the rats was less than those observed with 5 μg/kg. A single i.p. dose of 25 μg/kg given 24 hr after transplantation enhanced the host survival period. Twenty μg/kg s.c. given on 5 consecutive days had no inhibitory action on the tumor.

In Vitro Studies

Viable cell counts were 75% and 84% in treated and control groups of cells respectively. Rats injected with treated cells survived longer than controls, showing a T/C value of 168 (Table 2).

Yoshida Sarcoma. As with ascites tumor, 5 injections of 5 μg/kg given i.p. suppressed the growth of the tumor by 40%, while 5 s.c. doses of 20 μg/kg had no effect (Table 3).

Mouse Fibrosarcoma. The extract in 5 consecutive doses of 2 μg/kg i.p. and 5 μg/kg s.c. was inhibitory to tumor growth showing T/C values of 0.33 and 0.41 respectively (Table 3).

Cytomorphologic Effects on YAS Cells

Cytoplasm. Some of the cellular effects observed during the course of the treatment are shown in Figs. 1-6. Vacuolation and disruption of the cytoplasm, finally leaving behind naked nuclei, was a prominent feature in treated cells. This was observed in all smears taken after initiation of treatment and was encountered for 48 hours after stoppage. The intensity of cytoplasmic effect was maximum on the fourth day of treatment, as shown in Fig. 1. These features were prominent in tumor cells given 5 consecutive doses of 7.5 μg/kg, though they were also seen with lower doses.

Nuclei. Analysis of YAS cell smears stained with Feulgen's technic, from 24 to 144 hours after initiation of treatment,
were taken into consideration for evaluating the nuclear effects of the seed extract. Faint staining of the nuclei and karyolysis in nondividing cells is seen from 48 hours after starting the treatment (Fig. 2). Increased numbers of dividing nuclei with affected chromosome complement are found at 48 hours, followed by a gradual decline in mitotic stages in 72 hours and later smears. The decrease in the number of mitotic nuclei appear to be associated with constant increase in agglutinated and disintegrating chromosomes, as seen in Table 4. These abnormalities are depicted in Figs. 3-5. The cells in anaphase and telophase are comparatively fewer in number. A cumulative effect of the extract is seen after the treatment in which cells with disintegrated nuclei are seen in large numbers (Table 4, Fig. 6). These types of nuclei are observed in untreated cells also, but are fewer in number (5). Ascitic fluid drawn 3-4 days after completion of the treatment showed stem line cells with few degenerated and disrupted cells. The effect of the extract was seen at all doses, the intensity and duration being dose dependent.

Similar cytotoxic effects were observed in ascites tumor cells 24 and 48 hours after injection of a single dose of the extract to a well-established 5-day-old ascites tumor.

**DISCUSSION**

*A. precatorius* L. extracts have been screened by various workers for antitumor activity. Crude aqueous and ethanol seed extracts are reported to be noneffective on Sarcoma 180, adenocarcinoma 755, and lymphoid leukemia 1210 (2), while the crude aqueous extracts of root, stem, leaf, and fruit are found to be active against Sarcoma 180 and Lewis lung carcinoma, but not on lymphoid leukemia 1210 and KB (Eagle) tumors (1). This inhibitory activity could not be confirmed in a second experiment by the same investigators. Our studies reveal that a protein fraction of *A. precatorius* L. seeds (scarlet variety) in tolerated doses inhibits the growth of YAS and MFS with a lesser activity on YS. The results presented in this communication are those of the confirmatory experiment. The earlier studies had revealed the same trend of the results.

---

**Table 3**

<table>
<thead>
<tr>
<th>Tumor system</th>
<th>Dose* (mg/kg)</th>
<th>Total dose (mg/kg)</th>
<th>Route</th>
<th>Survivors</th>
<th>Weight difference (gm)</th>
<th>Tumor weight (gm)</th>
<th>TIC</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse fibrosarcoma</td>
<td>Control 2</td>
<td>10</td>
<td>i.p.</td>
<td>25/25</td>
<td>-1.2</td>
<td>2.99 ± 0.32</td>
<td>0.33</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Control 5</td>
<td>25</td>
<td>s.c.</td>
<td>25/25</td>
<td>-1.9</td>
<td>1.25 ± 0.14</td>
<td>0.41</td>
<td>59</td>
</tr>
<tr>
<td>Yoshida sarcoma</td>
<td>Control 25</td>
<td>5</td>
<td>i.p.</td>
<td>25/25</td>
<td>-2.3</td>
<td>3.87 ± 0.36</td>
<td>0.60</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Control 100</td>
<td>10</td>
<td>s.c.</td>
<td>25/25</td>
<td>-7.0</td>
<td>3.82 ± 0.38</td>
<td>0.94</td>
<td>6</td>
</tr>
</tbody>
</table>

*Effect of Abrus precatorius L. seed extract on experimental solid tumors.*

*Daily consecutive doses beginning day after implantation. Controls injected with saline.*

**Table 4**

<table>
<thead>
<tr>
<th>Time*</th>
<th>Prophase</th>
<th>Metaphase</th>
<th>Anaphase</th>
<th>Telophase</th>
<th>Stickiness</th>
<th>Agglutination</th>
<th>Disintegration</th>
<th>Scattering</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>3/8c</td>
<td>2/10</td>
<td>1/4</td>
<td>5/11</td>
<td>1/1</td>
<td>12/3</td>
<td>2/0</td>
<td>0/0</td>
</tr>
<tr>
<td>48</td>
<td>46/39</td>
<td>25/12</td>
<td>7/8</td>
<td>9/9</td>
<td>0/0</td>
<td>18/3</td>
<td>22/2</td>
<td>2/1</td>
</tr>
<tr>
<td>72</td>
<td>30/26</td>
<td>20/16</td>
<td>2/8</td>
<td>10/4</td>
<td>0/2</td>
<td>13/0</td>
<td>34/2</td>
<td>2/2</td>
</tr>
<tr>
<td>96</td>
<td>21/33</td>
<td>5/29</td>
<td>2/10</td>
<td>3/11</td>
<td>7/11</td>
<td>18/0</td>
<td>61/4</td>
<td>1/3</td>
</tr>
<tr>
<td>120</td>
<td>20/28</td>
<td>9/13</td>
<td>2/5</td>
<td>2/6</td>
<td>0/12</td>
<td>30/1</td>
<td>76/7</td>
<td>0/2</td>
</tr>
<tr>
<td>144</td>
<td>6/16</td>
<td>4/3</td>
<td>0/2</td>
<td>1/3</td>
<td>4/5</td>
<td>10/7</td>
<td>140/76</td>
<td>0/0</td>
</tr>
</tbody>
</table>

*Influence of Abrus precatorius L. seed extract on tumor cells in mitosis (Yoshida ascites sarcoma in vivo).*

*Hours after initiation of treatment.*

*Analysis based on a count of 2,000 cells.*

*Treated control; 5 consecutive daily doses of the extract, 7.5 mg/kg i.p. were used.*
A direct cytopathic effect appears to be one of the causes for the tumor inhibition. This is revealed by the morphologic changes seen in the cytoplasm and nuclei of the treated ascites cells both in vivo and in vitro. The extract influences both nondividing and dividing cells. Effect on nondividing cells is shown by karyolysis. The constant increase in chromosomal aberrations after treatment reveals the toxic action of the extract on cells in division. Faint staining with Feulgen's technic seen in treated cells indicates the effect of the extract on DNA content of the cell. The cytotoxic nature of seed extract has been demonstrated against KB cells also in tissue culture (4).

The extract appears to be more effective by i.p. route on YAS; multiple doses exhibited greater inhibitory activity than a single dose. The lesser activity and absence of inhibition by s.c. route in rats may be due to the slower absorption and lesser biophase concentration of the extract. Toxicity of the extract prevented trials at higher concentration by the s.c. route.

The cytotoxic effect on the cells is confirmed by a decrease in viable cell count after in vitro incubation of cells with the extract. There is the possibility that, though appearing viable by the dye test, the incapability of some of the treated cells to divide further might also play a role in enhanced survival period of hosts injected with in vitro incubated YAS cells.

Variations in the reports of earlier workers and our findings on the antitumor effects might be due to the different tumor systems employed and in the nature of the extract used. The semipurified protein extract used in our studies might be more potent than the crude extracts tested earlier.

Electrophoretic analysis shows the test material to be non-homogeneous and to consist of 3 distinct components. Isolation, characterization, and study of biologic activity of these fractions are in progress.

ACKNOWLEDGMENTS

The authors are thankful to Mr. V. B. Desai for helping in the preparation of the seed extract and to Mrs. C. R. Nagarathna Bai for technical assistance. One of the authors (V.V.S.R.) wishes to thank the authorities of the Indian Council of Scientific and Industrial Research for a fellowship award.

REFERENCES

Effect of *Abrus precatorius* L. on Experimental Tumors

V. V. Subba Reddy and M. Sirsi


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/29/7/1447

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.