Immunopotentiating activity of abrin, a lectin from *Abrus precatorius* Linn

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A non-toxic dose of abrin, (1.25 µg/kg body wt) consecutively for five days in normal mice stimulated specific humoral responses. A noticeable increase was observed in total leucocyte count, lymphocytosis, weights of spleen and thymus, circulating antibody titre, antibody forming cells, bone marrow cellularity and α-esterase positive bone marrow cells. The results suggest that abrin can potentiate the humoral immune response of the host.

Immunomodulators are being used in cancer therapy either in combination with chemotherapy or after chemotherapy and radiation therapy. Herbal preparations like Rasayanas used in indigenous system of medicines can enhance the body’s immune system\(^1\). A variety of materials from plant sources like polysaccharides, lectins\(^2\), proteins and peptides\(^3\) have been known to stimulate the immune system.

*Abrus precatorius* Linn which is commonly known as Rosary pea/ Jequiriti bean belonging to family Leguminosae and subfamily Papilionoideaec has a lectin known as abrin in its seeds. By using single step affinity column chromatography this lectin can be isolated from seeds\(^4\). Some of the important properties of abrin being toxic glycoprotein\(^5\), galactose specific\(^6\), haemagglutinating\(^7\), mitogenic\(^8\) and tumouricidal\(^9,10\).

It has been reported that toxic form of abrin gets converted to mitogenic form upon long refrigerated storage. When human lymphocyte cultures were incubated *in vitro* with abrin resulted in a strongly stimulated \(^3\)H thymidine uptake\(^11\). Abrin has also been widely used for construction of immunotoxins\(^12\). It was found to induce antitumour immunity against Meth-A tumour cells in mice\(^13\). The present study was undertaken to analyse the *in vivo* immunomopotentiating activity of abrin in normal mice.

**Materials and Methods**

Abrin was isolated from the seeds of red variety of *Abrus precatorius* using Sepharose 4B affinity col-

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umn chromatography and purified\(^14\) and the stock solution was stored at \(-20^\circ\)C. This solution was diluted with PBS to get a concentration of 250ng/ml. Inbred strains of Balb/c female mice (4-5 weeks old, 14-15g body weight) were purchased from Veterinary College, Mannuthy, Kerala and housed in well ventilated cages and fed with normal mouse chow (Lipton, India) and water *ad libitum*. Two groups of Balb/c mice (8 numbers/group) were taken and one group was treated with five doses of abrin (1.25 µg/kg body weight) and the other group (untreated control) was given 100 µl PBS on consecutive days.

Pararosanilin hydrochloride and α-naphthyl acetate were obtained from Loba chemie, Mumbai. Harris haematoxylin was purchased from Glaxo India Ltd., Mumbai. All other chemicals and reagents used were of analytical grade.

**Leucocyte counts**—Blood was collected from the caudal vein and parameters such as total leucocyte count (haemocytometer) and differential count (Leishman’s stain) were recorded prior to the drug administration and continued on every third day for 30 days.

**Organ weights**—Animals were sacrificed on the day after the last dose of abrin administration and the weight of vital organs such as liver, spleen, thymus and kidney were recorded and expressed as relative organ weights.

**Circulating antibody titre**—Two groups of Balb/c mice (8 numbers/group) were immunized with a single dose of non-trypsinised sheep red blood cells (20%; 100 µl) ip. One group of animals were injected (ip) with abrin (1.25 µg/kg body weight) on five days prior to immunization. Blood was collected from the caudal vein on every third day for one month. The
serum was separated and heat inactivated at 56°C. Antibody titre was determined by the haemagglutination method\textsuperscript{15} using SRBC.

**Plaque forming cells**—Two groups of Balb/c mice (8 animals/group) were immunized with a single dose of non-trypsinized SRBC (2.5\times10\textsuperscript{6}). One group of animals was administered (ip) with abrin (1.25\mu g/kg body weight) on five consecutive days prior to immunization. Animals were sacrificed on various days, spleens were processed and used for the plaque assay by the method of Jern\textsuperscript{16}.

**Bone marrow cellularity and \alpha-esterase positive cells**—Bone marrow cellularity was determined by the method of Mehra and Vaidya.\textsuperscript{17} The day after the last dose of drug, animals were sacrificed Bone marrow cells were collected from both femurs. The number of cells was counted and expressed as the total number of live cells/femur. From the above bone marrow cell preparation, smears were made on clean glass slides and stained with pararosaniline and haematoxyline to determine the nonspecific esterase activity\textsuperscript{18} by simultaneous azo dye coupling method.

Statistical analysis was carried out using Student’s \(t\) test and the results were expressed as mean \(\pm\) SD.

**Results**

**Leucocyte counts in normal mice**—There was a significant \((P<0.05)\) increase in the value of total leucocyte count in the abrin treated group (11294\pm1345 cells/\mu l) as compared to the value before treatment (7944\pm227 cells/\mu l). The absolute lymphocyte count of abrin treated group was significantly \((P<0.05)\) higher (9172\pm1360 cells/\mu l) as compared to before treatment (6021\pm275 cells/\mu l) value. There was a marginal increase in neutrophils observed during the period of study. (Fig. 1).

**Organ weights**—There was a significant \((P<0.05)\) increase in the organ weights of spleen and thymus of abrin treated group as compared to the untreated controls (Table 1).

**Circulating antibody titre**—Circulating antibody titres were increased in the abrin treated animals, compared to control group (Table 2). Maximum titre value of 512 was observed on 12\textsuperscript{th} day after antigen administration, while control animals showed a maximum titre value of 64 on 9\textsuperscript{th} and 12\textsuperscript{th} day.

**Plaque forming cells**—There was a significant increase in the number of plaque forming cells in abrin treated group of animals (Fig. 2). The number of plaque forming cells was increased to 965/10\textsuperscript{6} spleen cells (5\textsuperscript{th} day) in the treated group of animals whereas...
the maximum number of plaque forming cells in the untreated animals was only 420/10^6 spleen cells (4th day).

**Bone marrow cellularity and α-esterase positive cells**—Abrin treated group showed a significant *(P<0.05)* increase in the number of bone marrow cells (12.8±0.57 million/femur) compared to normal (11.78±0.42 million/femur). There was a significant *(P<0.05)* increase in the α-esterase positive cells in the abrin treated group (1085±54/4000 bone marrow cells) compared to control animals (893±75/4000 bone marrow cells).

**Discussion**

The body’s immunity has been shown to be suppressed in several diseases like AIDS and cancer. The

**Table 1—Organ weights**

[Values expressed as g/100g body wt are mean ± SD]

<table>
<thead>
<tr>
<th>Control</th>
<th>Liver</th>
<th>Spleen</th>
<th>Thymus</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.09±0.11</td>
<td>0.36±0.05</td>
<td>0.18±0.01</td>
<td>1.50±0.09</td>
</tr>
<tr>
<td>Abrin treated</td>
<td>4.21±0.22</td>
<td>0.46±0.03</td>
<td>0.20±0.01</td>
<td>1.55±0.07</td>
</tr>
<tr>
<td>t value</td>
<td>1.15</td>
<td>4.37</td>
<td>3.30</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Superscripts differ significantly *(P< 0.05)*.

**Chemotherapy and radiation therapy** in cancer treatment contribute to depress the immune system. Any imbalance occurring between regulatory and effector cells of immune system can also lead to immunological breakdown and pathogenesis. Use of immunomodulating agents can solve these problems to a greater extent.

Many immunomodulators such as levamisole, IFN, IL-2, *Corynebacterium parvum*, glucan, L-fucose etc are used in combination with cisplatin, adriamycin, 5-flourouracil etc. against many types of carcinomas. The greatest disadvantage with these synthetic immunomodulators is the side effects associated with them namely fever, myalgias, fatigue, neutropenia, anorexia, elevated serum transaminases and proteinuria. Plant based immunomodulators such as Rasayanam, extracts of *Viscum album*, extracts of *Withania somnifera*, extracts of *Tinospora cordifolia*, and curcumin from *Curcuma longa* could stimulate the immunity in normal and tumour bearing mice without any side effects.

The results of the present study indicate that the abrin isolated from *Abrus precatorius* significantly increased the total leucocytic count, lymphocytosis without causing neutropenia, weights of spleen and

![Fig. 2—Effect of abrin on plaque forming cells](image-url)
thymus, bone marrow cellularity and α-esterase positive cells. Abrin could potentiate the humoral immune response indicated by an increase in circulating antibody titre and the number of antibody forming (plaque forming) splenocytes. To summarize, administration of abrin at a dose rate of 1.25 μg/kg body weight, for 5 days in normal mice could stimulate specific humoral immune responses.

We have already reported that abrin could reduce the development of solid and ascites tumours in mice when used at a dose rate of 7.5 μg/kg body weight. At this concentration, abrin did not produce any noticeable variation in leucocyte counts, RBC counts, haemoglobin content and body weight and did not induce any organ injury. Immunomodulators like abrin at nontoxic concentrations and other natural products are thus strongly recommended for treating various immunopathological conditions including cancer.

References