Immunomodulatory Activity of the Ayurvedic Formulation
“Ashwagandha Churna”

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Abstract
Immunomodulatory activity was evaluated for “ashwagandha churna,” a reputed Ayurvedic herbal formulation. The experimental paradigms used were cellular (foot pad swelling) immune responses to the antigenic challenge by sheep RBCs (SRBCs) and the neutrophil adhesion test. On oral administration, ashwagandha churna showed a significant increase in neutrophil adhesion and delayed-type hypersensitivity (DTH) response. It is concluded that ashwagandha churna significantly potentiated the cellular immunity by facilitating the footpad thickness response to SRBCs in sensitized rats.

Keywords: Ashwagandha churna, delayed-type hypersensitivity (DTH), neutrophil adhesion.

Introduction
The use of plant products as immunomodulators is still in a developing stage. There are several herbs used in the indigenous systems of medicine that may modulate the body’s immune system. A variety of plant-derived materials such as polysaccharides, lectins, peptides, flavonoids, and tannins have been reported to modulate the immune system (Ielpo et al., 2000; Kuttan, 2000).

Rasayanas are a group of nontoxic herbal drug preparations that are used to improve the general health by stimulating the body’s immunity (Kumar et al., 1999). Churnas are preparations composed of fine powders of drugs and may be simple or compound. A simple churna consists of only one ingredient, and a compound churna consists of more than one ingredient. Churnas are prepared from powder of the dried roots of herbs. The efficacy of churnas depends on the use of fresh and genuine herbs and their careful compounding.

In the Ayurvedic system of treatment, churnas are as good as asavas and aristas for the eradication of diseases. The principle of using churnas is due to the fact that the therapeutic value of most of the substances greatly increases when they are reduced to very fine state of subdivision. They are also easily administered, especially in the cases of children who cannot swallow pills, tablets, or capsules.

Ashwagandha [Withania somnifera L. Dunal (Solanaceae)] churna is an Ayurvedic formulation that is popular as a home remedy for several diseases and human requirements (Patwardhan et al., 1988; Sharma & Dan-diya, 1991). It is also an official drug and is mentioned in the Indian Pharmacopoeia (Indian Pharmacopoeia, 1985). Regular usage of the ashwagandha churna is reported to purify the body and increase the life force (Rege et al., 1999).

In the current work, ashwagandha churna was evaluated for immunomodulatory effects. Such studies are important to substantiate the claims made with regard to the traditional formulations documented in ancient Ayurvedic texts.

Materials and Methods

Animals
Albino Wistar rats of the either sex (180–200 g) were used for the current study. They were maintained under standard environmental conditions and were fed standard pellet diet (Hindustan lever Ltd., Kolkata, India) and...
water *ad libitum*. Fresh sheep red blood cells (SRBCs) in Alsever’s solution were obtained from Hebbal Veterinary College (Bangalore, India).

**Antigen**

SRBCs collected in Alsever’s solution were washed three times in large volumes of pyrogen-free 0.9% normal saline and adjusted to a concentration of $0.5 \times 10^9$ cells/ml for immunization and challenge.

**Treatment**

Ashwagandha churna was purchased from the local market in Bangalore and used as received. The animals were divided into five groups consisting of six animals each. A group of six untreated rats were taken as control (group I). The Ashwagandha churna formulation was dissolved in water and fed orally for 14 days at a dose of 50 mg kg$^{-1}$ day$^{-1}$ (group II), 100 mg kg$^{-1}$ day$^{-1}$ (group III), 200 mg kg$^{-1}$ day$^{-1}$ (group IV), and 300 mg kg$^{-1}$ day$^{-1}$ (group V) for assessment of immunomodulatory effect.

**Delayed-type hypersensitivity (DTH) response (footpad swelling)**

Six animals per group (control and treated) were immunized on day 0 by i.p. administration of $0.5 \times 10^9$ SRBCs/rat and challenged by a subcutaneous administration of $0.025 \times 10^9$ SRBCs/ml into right hind footpad on day +14. The ashwagandha churna was administered orally from day −14 until day +13. DTH response was measured at 24 h after SRBC challenge on day +14 and expressed as mean percent increase in paw volume (plethysmometrically) (Puri et al., 1993).

**Neutrophil adhesion test**

On the 14th day of drug treatment, blood samples were collected (before challenge) by puncturing the retro-orbital plexus into heparanized vials and were analyzed for total leukocyte counts (TLC) and differential leukocyte counts (DLC) by fixing blood smears and staining with Field stain I and II–Leishman’s stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below (Wilkinson, 1978).

$$\text{Neutrophil adhesion (\%)} = \frac{\text{NI}_t - \text{NI}_u}{\text{NI}_u} \times 100$$

where NI$_u$ is neutrophil index of untreated blood sample, and NI$_t$ is neutrophil index of treated blood sample.

**Statistical analysis**

The data were analyzed using one-way analysis of variance (ANOVA) followed by Student’s *t*-test. *p* values < 0.05 were considered significant.

**Results and Discussion**

Ashwagandha churna Ayurvedic formulation showed significant increase in neutrophil adhesion (*p* < 0.05) at a dose of 300 mg kg$^{-1}$ day$^{-1}$ in rats. The results of neutrophil adhesion test are shown in Table 1.

The DTH response to SRBCs, which corresponds with cell-mediated immunity, showed a dose-dependent increase due to the treatment with ashwagandha churna. With doses of 200 and 300 mg kg$^{-1}$ day$^{-1}$, the DTH response was 9.58 ± 2.31 and 10.11 ± 1.33, respectively, in comparison with corresponding value of 5.65 ± 3.91 for untreated control group. The differences in DTH response were statistically significant (*p* < 0.05). The results of DTH responses are shown in Table 1. Thus, ashwagandha churna formulation treatment induced marked enhancement of DTH response to SRBCs in the animals.

Immunomodulatory agents of plant and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. In the current study, ashwagandha churna when orally administered significantly increased the adhesion of neutrophils.

**Table 1.** Effect of ashwagandha churna on neutrophil adhesion and DTH response to antigenic challenge by SRBCs in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Neutrophil index</th>
<th>DTH response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated blood</td>
<td>Fiber-treated blood</td>
</tr>
<tr>
<td>Group I (Untreated)</td>
<td>223.92 ± 35.22</td>
<td>190.56 ± 53.35</td>
</tr>
<tr>
<td>Group II (50 mg/kg, p.o.)</td>
<td>229.33 ± 68.19</td>
<td>195.61 ± 30.12</td>
</tr>
<tr>
<td>Group III (100 mg/kg, p.o.)</td>
<td>235.45 ± 18.61</td>
<td>197.33 ± 60.33</td>
</tr>
<tr>
<td>Group IV (200 mg/kg, p.o.)</td>
<td>255.59 ± 29.44</td>
<td>198.72 ± 70.19</td>
</tr>
<tr>
<td>Group V (300 mg/kg, p.o.)</td>
<td>265.38 ± 35.61</td>
<td>210.22 ± 21.68</td>
</tr>
</tbody>
</table>

DTH, delayed-type hypersensitivity; SRBCs, sheep red blood cells.
The values are mean ± SD of six rats in each group. One-way ANOVA followed by Student’s *t*-test; *p* < 0.05.
neutrophils to nylon fibers, which correlates with the process of margination of cells in blood vessels. The neutrophil adhesion was significantly increased with the dose of 300 mg kg\(^{-1}\) day\(^{-1}\) when compared with untreated control indicating possible immunostimulant effect.

The DTH response, which is a direct correlate of cell-mediated immunity (CMI), was significantly increased at a doses of 200 and 300 mg kg\(^{-1}\) day\(^{-1}\) of the ashwagandha churna Ayurvedic formulation. During CMI responses, sensitized T-lymphocytes, when challenged by the antigen, are converted to lymphoblasts and secrete lymphokines, attracting more scavenger cells to the site of reaction. The infiltrating cells are thus immobilized to promote defensive (inflammatory) reaction. In our studies, foot volume was enhanced after ashwagandha churna treatment suggesting cell-mediated immune enhancement (Sen et al., 1992). Increase in the DTH response indicates that ashwagandha churna has a stimulatory effect on lymphocytes and accessory cell types required for the expression of the reaction (Mitra et al., 1999).

On the basis of the results obtained in the current study, it can be concluded that ashwagandha churna has the potential to stimulate cell-mediated immunity and it may be a potential therapeutic candidate in several immunosuppressed clinical conditions. However, more exhaustive work needs to be performed to substantiate the claim.

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**References**


