Immunomodulatory activity of *Withania somnifera*

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Received 31 July 1999; received in revised form 8 November 1999; accepted 6 December 1999

Abstract

Administration of an extract from the powdered root of the plant *Withania somnifera* was found to stimulate immunological activity in Babl/c mice. Treatment with five doses of Withania root extract (20 mg/dose/animal; i.p.) was found to enhance the total WBC count (17 125 cells/mm³) on 10th day. Bone marrow cellularity (27 × 10⁶ cells/femur) as well as α-esterase positive cell number (1800/4000 cells) also increased significantly (*P* < 0.001) after the administration of Withania extract. Treatment with Withania extract along with the antigen (SRBC) produced an enhancement in the circulating antibody titre and the number of plaque forming cells (PFC) in the spleen. Maximum number of PFC (985 PFC/10⁶ spleen cells) was obtained on the fourth day. Withania extract inhibited delayed type hypersensitivity reaction in mice (Mantoux test). Administration of Withania extract also showed an enhancement in phagocytic activity of peritoneal macrophages (76.5 pigmented cells/200) when compared to control (31.5/200 cells) in mice. These results confirm the immunomodulatory activity of *W. somnifera* extract, which is a known immunomodulator in indigenous medicine. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: *Withania somnifera*; Bone marrow cellularity; Total WBC count; α-Esterase activity; Antibody titre; Plaque forming cells

1. Introduction

Immunosuppression is a major drawback in conventional therapy of cancer such as radiation and chemotherapy (Hersh and Freiriech, 1969). Both these methods have severe side effects such as nausea, vomiting, alopecia, mucosal ulceration, pulmonary fibrosis, cardiac and hepatic toxicity etc. Drugs that could alleviate these side effects will be highly useful in cancer therapy.

Use of plants as a source of immunomodulators is still in its infancy in modern medicine. Some of the plants with known immunomodulatory activities are *Viscum album*, *Panax ginseng*, *Tinospora cordifolia*, *Asparagus racemosus* etc. Components such as polysaccharides, lectins (Haijto et al., 1989) proteins and peptides (Kuttan and Kuttan, 1992) present in plants have been shown to stimulate the immune system.

*Withania somnifera* (L.) Dunal commonly known as Aswagandh (family Solanaceae) is extensively used in many indigenous preparations. *W. somnifera* is reported to have anti-inflammatory (Budhi-raja and Sudhir, 1998) antiarthritic (Sethi et al., 1990) and antitumour (Sbobat et al., 1998) activity.
1969) activities. Withania extract administration was found to increase the haemoglobin level, RBCs and decrease serum cholesterol, ESR etc. (Ziauddin et al., 1996). Administration of *W. somnifera* could reduce the lecopenia, enhanced the bone marrow cellularity and the ratio of normochromatic to polychromatic erythrocytes in mice treated with nonlethal dose of gamma radiation (Kuttan, 1996). Administration of Withania could also enhance the total WBC count, bone marrow cellularity as well as esterase positive cells in mice treated with cyclophosphamide (Davis and Kuttan, 1998).

No systematic studies have been reported to prove scientifically the immunomodulatory activity of Withania. In the present study, we report immunomodulatory activity of *W. somnifera* in normal Balb/c mice.

### 2. Materials and methods

Balb/c mice (males, 20 ± 3 g) were purchased from the National Institution of Nutrition, Hyderabad, India and the animals were kept in air controlled rooms and fed with normal mouse chow (Lipton, India) and water ad libitum.

Para-rosaniline hydrochloride and α-napthyl acetate were obtained from Loba Chemie, Bombay. Harris haematoxylin was purchased from Glaxo (Bombay, India). All other chemicals used were of analytical reagent grade. Sheep red blood cells (SRBC) was collected from local slaughter house in Alsevers solution.

#### 2.1. Drug preparation

Root from authenticated Withania plant was obtained from Amala Ayurveda Hospital and Research Centre Thrissur (Batch No. WS 031). The powdered root was extracted with 70% methanol by stirring at room temperature for 24 h. The supernatant was collected after centrifugation was evaporated to dryness in vacuo resuspended in phosphate-buffered saline (PBS) (pH 7.2) containing 1% gum acacia.

#### 2.2. Determination of effect of *Withania somnifera* on the haematological parameters

Balb/c mice (6 nos) were treated with five sublethal doses of Withania extract (20 mg/dose/animal, i.p.). Preliminary studies indicated that this dosage was found to maximally stimulate haemopoietic system and did not produce any toxicity (LD50 of Withania extract was 50 mg/dose/animal). Blood was collected from the caudal vein and parameters such as total WBC count (haemocytometer), differential count (Leishman’s stain), and haemoglobin level (cyanmethaemoglobin method) were recorded prior to the drug administration and continued on every third day for 30 days.

#### 2.3. Determination of the effect of *Withania somnifera* on organ weight of mice

Balb/c mice (6 Nos) were treated with five sublethal doses of Withania extract (20 mg/dose/animal, i.p.) for 5 days. Body weight of animals was recorded after the drug administration and animals were weighted and sacrificed after the last dose of drug treatment and the weight of vital organs such as liver, spleen, thymus, kidney were recorded and expressed as relative organ weights.

#### 2.4. Determination of effect of *Withania somnifera* on the bone marrow cellularity and α-esterase positive cells

Balb/c mice (6 Nos) were treated with Withania extract (20 mg/dose/animal i.p.) for 5 days. Animals were sacrificed after the last dose of drug treatment and bone marrow was collected from femur into medium containing 2% FCS. The number of bone marrow cells was determined using a haemocytometer and expressed as total live cells/femur. A smear of the bone marrow cells from the above preparation was made on clean glass slides and stained with Para-rosaniline hydrochloride and counter stained with haematoxylin to determine the non-specific α-esterase positive cells according to the method of Bancroft and Cook (1984).
2.5. Determination of effect of Withania somnifera on circulating antibody titre

Balb/c mice (12 nos.) were immunized with SRBC (0.1 ml, 20% i.p.) and divided into two groups — one group was treated with Withania extract (20 mg/dose/animal i.p.) each day for 5 days and the other group was kept as control. Blood was collected from the caudal vein prior to the antigen administration and continued every third day for 1 month. Serum was separated and heat inactivated at 56°C for 30 min. Antibody titre was estimated by the method of Singh et al. (1984) using SRBC as the antigen. For this, serum was serially diluted with PBS in 96 well round bottom titre plates. Equal volumes of 1% trypsinized SRBC (Ag) were added, mixed gently and incubated at room temperature for 3 h, and the agglutination titers were recorded.

2.6. Determination of the effect of Withania somnifera on the antibody producing cells

The number of plaque forming cells from the spleen was determined by the Jerne's Plaque assay (Jerne and Nordin, 1963). Balb/c mice (9 Nos) were immunized with SRBC (2.5 × 10⁸) in the presence and absence of Withania (20 mg/dose/animal, i.p.) one dose daily for 5 days prior to the antigen administration. The animals were sacrificed on different days; spleens were processed into a single cell suspension and the cell number was adjusted to 8 × 10⁶ cells/ml. Fifty microlitres of spleen cell suspension and 50 µl of Ag (SRBC 7%) were mixed with 0.5 ml of 0.5% molten agarose kept at 45°C and spread on slides. After solidifying the agarose, the gels were incubated in the presence of complement (rabbit serum at 37°C for 1 h). Number of plaques were counted using a colony counter.

2.7. Determination of the effect of Withania extract on delayed type hypersensitivity reaction (DTH)

DTH was measured by the Mantoux test. For this two groups of Balb/c mice (6 mice/group) were immunized with SRBC (1 × 10⁹/20 µl; i.p.). One group of animals were administered with five doses of Withania extract (20 mg/dose/animal, i.p.) for 5 days. After the fifth doses, the animals were injected with a challenging dose of antigen SRBC (1 × 10⁹/20 µl) on the left hind paw. DTH was determined by measuring the thickness of the paw after 24 h, according to the method of Langrange et al. (1974).

2.8. Determination of effect of Withania on the phagocytic activity of macrophages

Peritoneal macrophages were elicited with sodium caseinate in Balb/c (6 Nos) mice treated with five doses (20 mg/dose/animal i.p.) of Withania extract. Macrophages were harvested after 5 days and the phagocytic activity was determined using opsonized SRBC (Mehera and Vaidya, 1993) and the results compared with the macrophages isolated from the untreated control.

2.9. Statistical analysis

All the experiments were repeated twice and the results are expressed as mean ± S.D. Statistical evaluation of the data was done using students t-test.

3. Results

3.1. Effect of Withania somnifera on the haematological parameters

Administration of Withania extract increased the total WBC count (Fig. 1); the maximum count was observed on the 10th day (17 125 cells/mm³). There was no significant difference in the ratio of lymphocytes to neutrophils as well as haemoglobin level after treatment with Withania extract (data not shown).

3.2. Effect of Withania somnifera on organ weights

Effect of Withania administration on organ weight of mice is given in Table 1. There was a significant increase in the size and weight of the
thymus (0.30 g/100 g b.wt.) and spleen (1.5 g/100 g b.wt.) in the Withania-treated group as compared to controls (thymus 0.07 g/100 g b.wt.; spleen 0.30 g/100 g b.wt.). There was no significant change in the weight of kidney and liver after Withania administration.

Fig. 1. Effect of Withania somnifera on total WBC count. ■—■, Withania treated; ●—●, control.

Table 1
Effect of Withania somnifera on relative organ weights

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative organ weights (g/100 g body. wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
</tr>
<tr>
<td>Normal</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>Vehicle (Gum Acacia)</td>
<td>0.35 ± 0.07</td>
</tr>
<tr>
<td>Withania</td>
<td>1.55 ± 0.02*</td>
</tr>
</tbody>
</table>

* Animals were treated with five doses of Withania extract (20 mg/dose/animal).
* P < 0.001.
### Table 2
Effect of *Withania somnifera* on bone marrow cellularity and α-esterase activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bone marrow cellularity (cells/femur)</th>
<th>α-Esterase activity (No. of alpha esterase positive cells/4000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>$15 \times 10^6 \pm 1.1$</td>
<td>1189 ± 4.01</td>
</tr>
<tr>
<td>Vehicle</td>
<td>$14 \times 10^6 \pm 1$</td>
<td>1040 ± 5.8</td>
</tr>
<tr>
<td>Withania</td>
<td>$27 \times 10^6 \pm 1.2^*$</td>
<td>1800 ± 6.20*</td>
</tr>
</tbody>
</table>

* Treated animals received five doses of *Withania* extract (20 mg/dose/animal).
* $P < 0.001$.

3.3. **Effect of Withania somnifera on the bone marrow cellularity and α-esterase positive cells**

The effect of *Withania* administration on the bone marrow cellularity and α-esterase positive cells is given in Table 2. *Withania* treated group showed a significant increase in the bone marrow cells ($27 \times 10^6$ cells/femur) compared to normal ($15 \times 10^6$ cells/femur) animals. Moreover the number of α-esterase positive cells were also found to be increased significantly ($P < 0.001$) in the *Withania* treated group (1800/4000 cells) compared to controls.

3.4. **Effect of Withania somnifera on circulating antibody titre**

The effect of *Withania* administration on antibody titre is given in (Fig. 2). Maximum antibody titre value of 1/512 was observed on 13th day after treatment with *Withania* which was retained until the 27th day. Maximum titre value from normal animal was found to be 1/128 on the 18th day.

3.5. **Effect of Withania somnifera on antibody producing cells**

*Withania* extract administration was found to significantly ($P < 0.001$) enhance the number of antibody producing cells in spleen (Fig. 3). The maximum number of plaque forming cells in the *Withania* treated group (985 cells/10^6 spleen cells) was observed on the fourth day while control animals had a maximum 310 PFC/10^6 spleen cells.

3.6. **Effect of Withania somnifera on delayed type hypersensitivity reaction**

The effect of *Withania* extract on DTH is given in Table 3. *Withania* extract was found to inhibit the delayed type hypersensitivity reaction significantly ($P < 0.001$).

3.7. **Effect of Withania somnifera on phagocytic activity of peritoneal macrophages**

Administration of *Withania* could enhance the

![Fig. 2. Effect of *Withania somnifera* on circulating antibody titre.](image-url)
phagocytic activity of peritoneal macrophages

Table 4. Number of macrophages with engulfed SRBC were significantly increased ($P < 0.001$; 76.5/200 cells) in treated group compared to untreated group (31.5/200 cells).

4. Discussions and conclusions

Immunoregulation is a complex balance between regulatory and effector cell and any imbalance in the immunological mechanism can lead to

Table 3

Effect of *Withania somnifera* on delayed type hypersensitivity reaction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial paw thickness (mm)</th>
<th>Final paw thickness (mm)</th>
<th>Difference in paw thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.36 ± 0.01</td>
<td>0.62 ± 0.03</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Withania</td>
<td>0.36 ± 0.02</td>
<td>0.37 ± 0.01</td>
<td>0.013 ± 0.02*</td>
</tr>
</tbody>
</table>

*All the animals were sensitized with SRBC ($1 \times 10^8$) and treated animals received five doses of Withania extract (20 mg/dose/animal).

* $P < 0.001$. 
Table 4
Effect of *Withania somnifera* on phagocytic activity of peritoneal macrophages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of pigmented macrophages/200 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>31.15 ± 3.3</td>
</tr>
<tr>
<td>Treated</td>
<td>76.5 ± 4.3*</td>
</tr>
</tbody>
</table>

* Treated animals received five doses of Withania extract (20 mg/dose/animal).

* * P < 0.001.

Moreover, administration of the drug was found to inhibit the delayed type hypesensitivity in mice. This supports the reported anti-inflammatory activity of *Withania* extract (Budhi-raja and Sudhir, 1998).

*W. somnifera* has been shown to contain Withanolides and steroidal lactones. At present we do not know whether these compounds are responsible for the immunostimulatory activity produced by this extract. Further studies using isolated compounds are in progress.

**Acknowledgements**

This work was supported by a grant from Gufic, Bombay.

**References**


