EXPERIMENTAL STUDY ON IMMUNOMODULATORY ACTIVITY OF NAGBALA (SIDA HUMILIS CAV)

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ABSTRACT

Background and aim: Nagbala is mentioned as Rasayana drug and used in treatment of Kasa, Raktpitta Kshata and Kshaya in classical text. Three plant species are considered as Nagbala by different scholars, Sida spinosa L., Sida humilis cav. and Grewia hirsuta Vahl. Out of these Sida humilis is selected as Nagbala to evaluate its immunomodulatory activity. So, the present study was aimed at evaluating the immunomodulatory activity of Nagbala (Sida humilis Cav.) roots by using Choorana and Kwath of roots in DTH model.

Methodology: Collection, identification and authentication of drug was done. Pharmacognostical study containing organoleptic, microscopic and physical has been performed. In experimental study Immunomodulatory activity was performed on Swiss Albino Mice DTH model, using choorna and Kwath of Drug orally. Results: Study of Immunomodulatory activity defined that Nagbala (Sida humilis Cav.) shows immunomodulatory activity by showing immune response to SRBCs by increasing paw volume in mice. Immunomodulatory activity of drug was compared to disease control group. Interpretation: By the experimental study it is cleared that Nagbala (Sida humilis Cav.) shows immunomodulatory activity but Kwath shows more immune response than powder of Nagbala roots.

KEYWORDS: Nagbala, Sida humilis Cav., immunomodulatory activity.

INTRODUCTION

The immune system is a system of biological structures and processes within organism that protects against disease. Disorders of immune system can result in autoimmune diseases and
cancer and immunodeficiency.\[1\] Immunomodulation is the procedure which can alter the immune system of an organism by interfering its functions; if it results in an enhancement of immune reaction, it is named as an immunostimulative drug which primarily implies stimulation of non-specific system. Immunosuppressant implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be considered in order to regulate the normal immunological functioning. Hence both immunostimulating agents and immunosuppressing agents have their own standing, so search for better agents exerting these activities is becoming the field of major interest all over the world. A number of Indian medicinal plants and various Rasayana have been claimed to possess immunomodulatory activity.\[2\]

Rasayana is branch of Ayurveda deals with prevention of diseases, promotion of healthy life, intelligence and strength. Modulation of immune responses to alleviate the diseases has been interest for many years & concept of Rasayana in Ayurveda is based on related principles. Many single drugs are described as Rasayana in Ayurvedic Samhitas. These drugs may be interpreted as life promoters & expected to act as immunopromoters also. This relation between Rasayana drug & immunomodulatory activity is explained in various research work.

Nagbala is single drug Rasayna mentioned in Brihattrayi. They had used Nagbala in both preventive aspect i.e. Rasayna Chikitsa and curative aspect i.e. treatment of various diseases. Also it is used as Naimittik Rasayana in Kshata and Kshaya. Sushruta Samhita mentioned it in Sarvopaghatashamaniya Adhyaya which suggests its property in healing diseases.\[3\] Charak Samhita had mentioned its specific details about collection administration etc points towards its vital place in Rasayana Chikitsa.\[4\]

There are three plant species considered as nagbala by different scholars Sida spinosa, Sida humilis, and Grevia hirsuta. From which Sida humilis is considered as Nagbala by Dr Chunekar Pande, Dr Balwant Singh Thakur and Dr Yadavji Trikamji Acharya. Also dr Yadavji Trikamji Acharya has explained meaning of Nagbala as plant which moves like a snake on ground which can be correlated to Sida humilis as it is a prostrate herb.\[5,6,7\]

Here Sida spinosa and Sida humilis belongs to same genus and also they have some same alkaloids and constituents. Sida spinosa had already studied for immunomodulatory activity by DTH method.\[Study of Rasayana Karma and immunomodulatory activity of Nagbala\]
(Sida spinosa) (water extract and ethanol extract were used.) by Dr. Kataria, Guide- Dr. Khilari 2003 B.V. D. U. College of Ayurved, Pune][8] In same way to determine whether Sida humilis possesses the same activity as Sida spinosa further attempt has been made to scientifically evaluate and validate the immunomodulatory effect of Nagbala.

MATERIALS AND METHODS

Plant material

Plant of Sida humilis Cav. Family Malvaceae collected from Pen Raigad in January 2015 and was authenticated from Department of Botany Agharkar institute, Pune. (Voucher specimen No.28870)

Preparation of dosage forms

Roots of Nagbala (Sida humilis Cav.) were used in this study. Two dosage forms Kwath (decoction) and Choorna (powder) of Nagbala roots were used for treatment. These dosage forms were prepared according to guidelines given in Sharangdhar Samhita. Powder was of 100 mesh size and Kwath was prepared fresh daily.

Experimental animals

Albino mice of either sex were used. The animals were feed with standard normal diet and maintained under standard environment condition employed. They were housed under standard normal conditions (22 ± 5ºC with 12 h of light/ dark cycles). All experiment protocols were approved by Institutional Animal Ethical Committee (55/1415) National toxicology Center, Pune.

Antigen

Fresh Sheep blood was collected in sterile Alsevar’s solution (1:1 proportion). Sheep red blood cells(SRBCs) were washed three times in pyrogens free normal saline and centrifuged at 2500- 3000 rpm for 10 minutes. The supernatant was removed with pasture pipette and suspended in normal saline. The concentration of 0.1 ml containing 1×10^8/mm³ cells was adjusted by using improved Neubar chamber for immunization and challenge.

Delayed type hypersensitivity test

The method described by H.G.Vogel was adopted.[9] Mice were divided into 5 groups of six mice each. Drug was administered in various group, group 1 (DC) was not given any treatment drug. Group 2(T1A) received powder dose (1300mg/kg) group 3 received powder
dose (650mg/kg), group 4 received decoction (10.4 ml/kg) and group 5 received decoction (5.2 ml/kg). All the mice are sensitizes with $1 \times 10^8$ SRBCs through SC route in left hind paw on day 0. All the groups were treated as per table.

All the animals were maintained on same diet and environment throughout the duration of experiment.

Administration of dosage forms were done by oral route by using animal feeding needle and 1 ml syringe. On day 6 the left hind paw thickness was measured. Animals were challenged with the same antigen $1 \times 10^8$ SRBCs in 0.1 ml; in the left hind paw by SC route.

The paw thickness measurement was again taken at the interval of 24th and 48th hr from challenge.

Table 1: Experimental Design

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Group 1-5</td>
<td>Sensitization with $1 \times 10^8$ SRBCs</td>
</tr>
<tr>
<td>Day 1-6</td>
<td>Group 2</td>
<td>1300mg/kg Dose of powder</td>
</tr>
<tr>
<td>Day 1-6</td>
<td>Group 3</td>
<td>650mg/kg Dose of powder</td>
</tr>
<tr>
<td>Day 1-6</td>
<td>Group 4</td>
<td>10.4ml/kg dose of decoction</td>
</tr>
<tr>
<td>Day 1-6</td>
<td>Group 5</td>
<td>5.2 ml/kg Dose of decoction</td>
</tr>
<tr>
<td>Day 6</td>
<td>Group 1-5</td>
<td>Measurement of paw thickness</td>
</tr>
<tr>
<td>Day 6</td>
<td>Group 1-5</td>
<td>Challenge with $1 \times 10^8$ SRBCs</td>
</tr>
<tr>
<td>Day 7-9</td>
<td>Group 1-5</td>
<td>Measurement of paw thickness</td>
</tr>
<tr>
<td>Day 10</td>
<td>Group 1-5</td>
<td>Collection of blood for WBC</td>
</tr>
</tbody>
</table>

On day 10, blood was collected from retro orbital plexus for WBC count.

Statistical analysis

The mean + SEM was calculated. The variation present in the data was analysed through one way analysis of variance (ANOVA). Post-ANOVA analysis was done by the Tukey’s multiple tests to estimate the significance of difference between various individual groups.

RESULTS

Effects of Nagbala (*Sida humilis* Cav.) on mean foot pad oedema in DTH model.

The results obtained in DTH model are given in table 2.

The immune response was evaluated by DTH response i.e. increase in foot pad thickness using vernier callipers. The observations in table 2 shows that mice treated with powder and
Decoction of Nagbala roots with all dose levels increase response in foot pad oedema was found to be statistically significant (p<0.05) when compared to group 1.

**Results of Nagbala (Sida humilis Cav.) on Haematological parameters. (Table 3)**

Administration of Drug in powder form shows statistically significant (p<0.05) increase in WBC count when compared to control group.

**Table 2: effect of Nagbala (Sida humilis Cav.) on mean foot pad oedema**

<table>
<thead>
<tr>
<th>Group</th>
<th>Group description</th>
<th>Mean foot pad oedema (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hr</td>
</tr>
<tr>
<td>1</td>
<td>DC</td>
<td>0.5±0.15</td>
</tr>
<tr>
<td>2</td>
<td>T1A</td>
<td>1.06±0.20</td>
</tr>
<tr>
<td>3</td>
<td>T1B</td>
<td>1±0.15</td>
</tr>
<tr>
<td>4</td>
<td>T2A</td>
<td>1.4±0.06</td>
</tr>
<tr>
<td>5</td>
<td>T2B</td>
<td>1.14±0.06</td>
</tr>
</tbody>
</table>

Data was expressed as mean + SEM, n=6; using one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test p<0.05 was considered as statistically significant, n=6 in each group. DC- diseased control, T1A- Powder dose 1, T1B- Powder dose 2, T2A- Kwath dose 1, T2B- Kwath dose 2.

**Graphical presentation effect on foot pad oedema**

**Table 3: effects of Nagbala (Sida humilis Cav.) on WBC count**

<table>
<thead>
<tr>
<th>Group</th>
<th>Group description</th>
<th>WBC count (Thousand /mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DC</td>
<td>7.73±2.4</td>
</tr>
<tr>
<td>2</td>
<td>T1A</td>
<td>11.38±1.5</td>
</tr>
<tr>
<td>3</td>
<td>T1B</td>
<td>10.6±1.3</td>
</tr>
<tr>
<td>4</td>
<td>T2A</td>
<td>10.96±1.6</td>
</tr>
<tr>
<td>5</td>
<td>T2B</td>
<td>10.3±1.9</td>
</tr>
</tbody>
</table>
Data was expressed as mean + SEM, n=6; using one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test p<0.05 was considered as statistically significant, n=6 in each group. DC- diseased control, T1A- Powder dose 1, T1B- Powder dose 2, T2A- Kwath dose 1, T2B- Kwath dose

Graphical presentation of WBC count

DISCUSSION

Rasayna drugs described in Ayurveda may possess immunomodulatory, antiageing, antioxidant, rejuvenating effects. Also they can prevent diseases and promotes healthy life. Nagbala is described as single drug Rasayana in Brihattrayi. For screening of immunomodulatory activity of Nagabala DTH method is used.

DTH is a part of the process of graft rejection, tumour immunity and most important immunity to many intracellular infectious microorganisms especially those causing chronic diseases such as tuberculosis.\(^{[10]}\)

DTH requires a specific recognition of given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. These in turn increase vascular permeability, induced vasodilatation, macrophage accumulation and activation, promoting increased phagocytic activity and increased concentration of lytic enzyme for more effective killing.\(^{[11, 12]}\)

Results of screening of immunomodulatory activity show that a group treated with high dose of Kwath (human dose 80 ml), immunity response is highest & slightly lower in high dose of Choorna(human dose 10 gms). Groups treated with low dose of Kwath and Choorna show
lower response than above two groups. Hence it is seen that groups treated with doses as per given in antient text show more response.

Immunity response is significantly low in disease control group than in treated group at 24 hr. It remains stable for 48 hrs or decreases slightly. In haematological parameters WBC count is supportive parameter for immunity response. WBC count show significant difference between disease control group & groups treated with Nagbala. These results have revealed that treated groups show more immune response. Nagbala Kwath and Nagbala Choorna both groups show difference in immune response. It is suggestive of that there may be difference in % of active constituents or difference in constituents in water extract (Kwath) and whole drug powder (Choorna).

DTH response is direct correlated to cell mediated immunity and was significantly increased with powder and decoction of Nagbala (Sida humilis Cav.) roots as compared to control group. The significant increase in Immunomodulatory potential of powder and decoction of Nagbala roots could be attributed due to presence of alkaloidal constituents viz. Beta-phenethylamines, quinazolines and carboxylated tryptamines, choline, betain starch, amino acids and lignin., cryptolepine and ephedrine\(^{13,14}\). Thus present study validates that traditional use of Nagbala (Sida humilis Cav.).

**CONCLUSION**

Experimental study clears that Rsayana drug Nagbala (Sida humilis Cav.) shows significant immunomodulatory activity.

Kwath and choorna both can be used for immunomodulatory activity, but Kwath kalpana is more effective.

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