Pharmacological Research

Immunomodulatory activity of Vachadhatryadi Avaleha in albino rats

Rajagopala S.1, Ashok B. K.2, Ravishankar B.3

1Assistant Professor, Department of Kaumarabhritya, 2Research Assistant, Pharmacology Laboratory, 3Ex. Head, Pharmacology Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

The present study is carried out to evaluate the immuno-modulatory activity of Vacha Dhatryadi Avaleha in albino rats. Vacha Dhatryadi Avaleha was prepared by classical method and evaluated for humoral antibody formation and cell-mediated immunity in established experimental models. Test formulation was administered at the dose of 900 mg/kg and parameters like hemagglutination titer, ponderal changes, histopathology of immunological organs and immunological paw edema were recorded. Vacha Dhatryadi Avaleha significantly enhanced antibody formation and moderately suppressed the immunological edema. The present study concludes that Vachadhatryadi Avaleha has immunopotentiating activity.

Key words: Cell mediated immunity, hemagglutination titer, Vachadhatryadi

Introduction

Ayurveda is the art and science of life, and is one of the richest heritages gifted to mankind by our great ancestors. One of the main strategies in Ayurvedic medicine is to increase body’s natural resistance to the disease-causing agent rather than directly neutralizing the agent itself in practice. Ayurveda has propounded the concept of immunity as "Vyadhikshamatwa".[1]

The use of herbs for improving the overall resistance of body against common infections and pathogens has been a guiding principle of Ayurveda[2] and for this there is a separate class of immunomodulatory drugs known as Rasayanas.[14] They are supposed to have the ability of protecting the body against external factors that induce disease. This implied resistance against disease may represent the modern concept of immunity.[7]

Prakara Yoga (indigenous method or practice of enhancing body immunity) narrated in the 35th chapter of text Arogya Raksha Kalpadrumam,[3] in which the drug schedule starts with birth and continues to the age of 12 years for the purpose of enhancing non-specific immunity of the body. In whole regimen of Prakara Yoga, good numbers of drugs are used at various developmental stages in which some drugs were found to be repeated at almost every level. The drugs which are used several times in this schedule and which are reported to have immunomodulatory activity were selected and a Avaleha (Confection) form was prepared for the clinical trials on children (Avaleha formulation being ideal for administration in children as the question of palatability and acceptability are made easier). Thus prepared formulation was subjected to pharmacological screening in experimental models of immunomodulatory activity to provide experimental basis to clinical findings.

Test formulations

Vachadhatryadi Avaleha contains Vacha (Acorus calamus Linn.), Dhatri (Emblica officinalis Garten.), Musta (Cyperus rotundus Linn), Pushkaramula (Inula racemosa Hook.), Jeeraka (Cuminum cuminum Linn.), Shankhapushpi (Convolvulus pluricaulis Chois.), Pippali (Piper longum Linn.), Sita (sugar), Kshaudra (honey), Sarpi (ghee) and Trikatu (three pungent drugs viz. powders of Shunti (Zingiber officinale Roxb), Maricha (Piper nigrum Linn.) and Pippali (Piper longum Linn.).[9] The combination was named after the first drug of the combination as Vachadhatryadi Avaleha.

The Adjuvant Yoga comprised the following drugs except the herbal drugs of the Vachadhatryadi Avaleha i.e., Kshaudra (honey), Sarpi (ghee), Sita (sugar) and Trikatu as Prakeshepa. This combination is formulated in order to elicit the action of the drugs under trial or to separate the action of Sita, Kshaudra and Sarpi, which are used as media for the preparation of Avaleha in Vachadhatryadi Avaleha formulation.

These two formulations (Avaleha) were prepared in Pharmacy attached to the institute as per classical procedures[10] and stored in an air tight container for experimental purposes.
Animals
Charles Foster strain albino rats of either sex weighing between 180 ± 30g were selected from the animal house attached to the institute. They were housed at 22 ± 03°C with constant humidity of 50-70% on a 12 h natural day and night cycles. They were fed with diet Amrut brand rat pellet food supplied by Pranav Agro Industries, Baroda and tap water was given ad libitum. The experiments were carried out in accordance of the Institutional animal ethics committee after obtaining its permission.

Dose selection and schedule
The classical dose of Vachadhatryadi Avaleha is 10 g/day. The dose for experimental animals was calculated by extrapolating the human dose to animals (900 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes. The drug solutions were made with distilled water (90 mg/ml) and administered to animals in the dose of 1 ml/100 g body weight with the help of gastric catheter sleeved to syringe. The drugs were administered to overnight fasted animals.

Effect on humoral antibody formation
The effect of test drugs on antibody formation against sheep red blood cells (SRBC) was studied as described by Puri et al. The selected animals were divided into three groups. First group received distilled water and served as the control group. Second group received Vachadhatryadi Avaleha and third group received Adjuvant Yoga. The drugs were administered for 10 consecutive days. On third day, sheep blood was collected from the city slaughter house in a sterilized bottle containing Alsever’s solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride) aseptically so that agglutination of blood does not take place. The collected sheep blood was thoroughly washed with sterile normal saline through repeated centrifugation until the supernatant fluid became colorless and made to 30% SRBC solution. This sensitizing agent was injected subcutaneously in the dose of 0.5 ml/100 g body weight to the rats.

On the eleventh day, the animals were sacrificed by ether over dose and the blood was collected in sterile test tubes. Serum was separated from it and complement in it was inactivated by taking its permission. and Barnes. [11] The drug solutions were made with distilled water (90 mg/ml) and administered to animals in the dose of 1 ml/100 g body weight with the help of gastric catheter sleeved to syringe. The drugs were administered to overnight fasted animals.

Estimation of antibody titer
The micro-titer plate was filled with 0.1 ml sterile normal saline and serial two fold dilutions of 0.1 ml of the serum in sterile saline solution were made in the micro-titer plate up to 16 times. 0.1 ml of thrice saline washed with 3% SRBC was added to each well of the tray. Blood from the same animal (sheep) was used for both sensitization and to determine antibody titer. The trays were covered and placed in refrigerator overnight. Antibody titer (hemagglutination titer) was noted on the next day. The titer was converted to log2 values for easy comparison.

Spleen, thymus and lymph nodes were dissected out from the animals and their weight was recorded. Tissues were transferred to 10% formaldehyde solution for fixation and the histopathological slides were prepared by referring standard procedure. The slides were viewed under binocular research Carl-Zeiss’s microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Effect on cell-mediated immunity
Effect on cell-mediated immunity was evaluated by following the procedure of Bhattacharya. [13] First group received distilled water and served as the control group. Second group received Vachadhatryadi Avaleha and third group received Adjuvant Yoga. All the animals were sensitized subcutaneously (0.5 ml/100 g body weight) on first day of drug administration by following solution; triple antigen (DPT) - 1ml, normal saline (0.9%) - 4 ml and potash alum (10%) - 1 ml. The pH of the above solution was maintained between 5.6 and 6.8 using 10% sodium carbonate. The drug administration was continued for seven consecutive days. On the seventh day, 1 h after drug administration the initial paw volume of left hind paw was noted and 0.1 ml of above solution was injected into plantar aponeurosis of same paw. Volume of immunological edema thus produced was measured by volume displacement method after 24 h and 48 h of injection with plethysmograph. Percentage increase in paw volume, which is the index of edema formation over initial value, was calculated. The values from control group were compared with the values from test and adjuvant drug administered groups to assess the cell-mediated immunity response of the drugs.

Statistical analysis
Results were presented as Mean ± SEM, difference between the groups was statistically determined by unpaired Student’s ‘t’ test with the level of significance set at P<0.05. The level of significance was noted and interpreted accordingly.

Results
The data on the effect of test drugs on body weight gain changes in experimental animals are shown in Table 1. In comparison to the control group where the percentage increase of body weight was 19.63%, the rate of increase in body weight was less in Vachadhatryadi Avaleha (8.33%) and Adjuvant Yoga (13.93%) treated groups. The decreased rate of body weight gain observed in Vachadhatryadi group was found to be statistically significant (P< 0.001) in comparison to body weight gain in control group.

Both Vachadhatryadi Avaleha and Adjuvant Yoga significantly (* * *P< 0.001) enhanced antibody titer [Table 2] in comparison to control group, the percentage of increase in Adjuvant Yoga (29.39%) was comparatively less as compared to the Vachadhatryadi Avaleha treated group (32.32%).

The data on the effect of test drugs on triple antigen induced immunological edema have been presented in Table 3. In Vachadhatryadi Avaleha administered group an apparent but statistically non-significant decrease in the paw volume was observed with respect to both 24 and 48 h recordings. In Adjuvant Yoga administered group no apparent effect was observed.

Discussion
A normal immune system develops through the interaction of many cellular and humoral components that develop at
different rates during fetal and early postnatal life.\(^{15}\) The main objective of Rasayana therapy is to equip the body in such a manner that it is toned up to combat its exposure to adverse conditions. The main mechanism is to enhance different body defense mechanisms in a non-specific manner to endowed with better capacity to get adapted to different kinds of adverse conditions.\(^{16}\) Among these effects is the enhancement of immune responsiveness of an organism against a pathogen by non-specifically activating the immune system using immunomodulating agents of plant origin.

Vachadhatryadi Avaleha and Adjuvant Yoga significantly (\(P<0.001\)) increased the antibody titer. This clearly shows that adjuvant Yoga (viz. comprised of Kshaudra (honey), Sarpi (ghee), Sita (sugar) and Trikatu as Prakshhepa) per se have anti-body enhancing effect [\(P<0.001; \text{Table 2}\). Combining adjuvant with primary drugs leads to further moderate increase in anti-body formation. In comparison to control group, adjuvant-administered group shows significant enhancement in anti-body titer (29.39%) and combination of Vacha, Dhatri, Musta, Pushkaramula etc. drugs with adjuvant further enhanced the anti-body formation (32.32%) [\(\text{Table 2}\). The possible stages for this can be accelerated processing of antigen by the macrophages, enhanced secretion of cytokines like IL-\(\gamma\) and tissue growth factor-b (TGF-b) both of which stimulate B-lymphocytes to proliferate.\(^{20}\) The other possible mechanisms can be enhanced response to the effect of cytokines released during the induction and proliferative phases possibly through up-regulation of the receptors involved, modulation of cytokine gene expression. Further both the formulations did not have any significant influence on the ponderal and histopathology of organs related to immune system.

Vachadhatryadi Avaleha decreased the immunopological paw edema both at 24 and 48 h which indicates that the formulation has only weak to moderate CMI suppression effect. Adjuvant Yoga did not produce any desirable effect on the CMI.

Many of the drugs used in this formulations are reported to have immune-modulatory activity; viz., Vacha,\(^{21}\) Dhatri,\(^{22-24}\) Musta,\(^{25,26}\) Pushkaramula,\(^{27}\) Pippali,\(^{28,29}\) Shunti\(^{30}\) and Maricha.\(^{31}\) Further, the immune-potentiating properties of Madhu\(^{32,33}\) and Ghrita\(^{34,35}\) are also well established. Thus, the observed activity may be attributed to one or more bioactive principles present in these drugs. From this study, it can be concluded that the Vachadhatryadi Avaleha has immunopotentiating activity and can be used to enhance the immune system in children.

### Acknowledgments

Authors are thankful to Director, IPGT and RA, Gujarat Ayurved University, Jamnagar for providing essential technical and financial support for this study.

### References

1. Agnivesha, Charaka Samhita, commentary by Chakrapani ‘ayurveda Dipika’, edited by Vaidya Jadavaji Trikamji Acharya, Chaukhambha Sanskrit Sansthan. Varanasi: reprint 2004, Sutrasthana, chapter 28, shloka 07.
2. Patwardhan B, Warude D, Pushpangadan P. Bhatt N. Ayurveda and traditional Chinese medicine: A comparative overview. Evid Based Complement Alternat Med 2005:2;465-73.
3. Bhaataracharya SK, Muruganandam AV. Adaptogenic activity of Withania somnifera - an experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 2003;75: 547-55.
4. Agnivesha, Charaka Samhita, commentary by Chakrapani ‘ayurveda Dipika’, edited by Vaidya Jadavaji Trikamji Acharya, Chaukhambha Sanskrit Sansthan. Varanasi: reprint 2004, Chikitsasthana, chapter 01, shloka 1/4, 1/7-8, 1/23, 4/6.
5. Sushruta, susruta Samhita, commentary by Sri Dalhanacharya ‘Nibandhasangraha’, chaukhambha Orientalia. chapter 27 and 30. 8 th ed. Varanasi, India: Chikitsasthana, 2005.
6. Vahata, Astangasamgraha, commentary by Indu, ‘sasilekha’, Chowkhamba Sanskrit series office. Varanasi, India: Uutta Tantra; chapter 49, shloka 1, 2.
7. Singh RH, Udupa KN. Clinical and experimental studies on Rasayana drugs and Rasayana therapy. In: Bhattacharyaa SK, Muruganandam AV. Adaptogenic activity of Withania somnifera - an experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 2003;75:547-55.
8. Kaikulangara Variath Rama Varier: ‘Arogyarakshakalpadrumam’. Chapter
इम्यूनोमोडुलेटरी एक्टिविटी ऑफ वचाधात्म्यादि अवलेह इन एल्बिनो रेट्स

राजगोपाल एस., अशोक बी. के., रविशंकर बी.

प्रस्तुत शोधकार्य में वचाधात्म्यादि अवलेह की व्याधिकाम्यता पर प्रभाव का प्रयोगिक अध्ययन अबलेह की जाति के चूहों पर किया गया।

इस अध्ययन में वचाधात्म्यादि अवलेह का हुमेइयसन एंड एवं सेल फिल्टरिंग इम्यूनोमोडुलेटरी पर प्रभाव का आध्ययन किया गया।

शास्त्रीय विद्या से मिलता है, परिसंचरण अवलेह ९० मिन. प्रति किलोग्राम शरीर की मात्रा में गुप बी के चूहों को खिलाया गया में और इसके प्रभाव का (किर्षलिप्ती टैक्टर) सक्रियता को नजर में परिवर्तन, क्योंकि आयुष्मान उत्तराधिकारी के शरीर पर प्रभाव का आध्ययन किया गया।

वचाधात्म्यादि अवलेह का शरीर में एंडोबोडी निर्माण को बढ़ाने में अवश्य प्रभावी रहा एवं इम्यूनोलोजिकल एडिका को घटाने में मध्यम रूप से प्रभावी रहा। इस अध्ययन के निष्कर्ष नुसार वचाधात्म्यादि अवलेह में व्याधिकाम्यता बढ़ाने की क्षमता है।