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Immunomodulatory activity of *Vachadhatryadi Avaleha* in albino rats

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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-medicated 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*.[3-6] 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*,[8] 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.

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Test formulations

Vachadhatryadi Avaleha contains *Vacha* (*Acorus calamus* Linn.), *Dhatri* (*Emblica officinalis* Garten.), *Musta* (*Cyperus rotundus* Linn), *Pushkaramula* (*Inula racemosa* Hook.), *Jeeraka* (*Cuminum cyminum* 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* formulaiton.

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 between180 ± 30g were selected from the animal house attached to the institute. They were housed at 22 ± 03ºC 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.[10] 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.[11] The drug solutions were made with distilled water (90mg/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 anti-body formation against sheep red blood cells (SRBC) was studied as described by Puri *et al.*[12] 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 of 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 incubating it for 30 min at 56°C temperature in a serological water bath.

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 $\log_2$ values for easy comparison.[13]

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.[14] 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.[15] 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[16] 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[17] 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

Table 1: Effect of *Vachadhatryadi Avaleha* and Adjuvant *Yoga* on ponderal changes in SRBC sensitized rats

Groups	Initial body weight	Final body weight	% increase in body weight	Thymus weight (g/100g)	Spleen weight (g/100g)
Control	168.33 ± 7.92	200.00 ± 6.32	19.63 ± 4.41	0.396 ± 0.044	0.447 ± 0.072
Vachadhatryadi Avaleha	201.67 ± 8.72	216.67 ± 9.89	08.33 ± 2.22	0.369 ± 0.024	0.323 ± 0.042
Adjuvant yoga	196.00 ± 14.47	221.67 ± 12.22	13.93 ± 2.52	0.357 ± 0.016	0.303 ± 0.017

The test formulations were administered daily by the oral route to groups of rats (n=6) at 900 mg/kg dose for 10 days. The data are expressed as Mean ± SEM

Table 2: Effect of *Vachadhatryadi Avaleha* and adjuvant Yoga on hemagglutination titer in SRBC pre-sensitized rats

	Hemagglutination titer (log 2 values)	% change
Control	3.93 ± 0.15	-
Vachadhatryadi Avaleha	5.20 ± 0.16***	32.32 ↑
Adjuvant yoga	5.09 ± 0.23***	29.39 ↑

The test formulations were administered daily by the oral route to groups of rats (*n*=6) at 900 mg/kg dose for 10 days. The data are expressed as Mean ± SEM. Significant differences in treated groups *vs* control group is ***P< 0.001

Table 3: Effect of *Vachadhatryadi Avaleha* and adjuvant *Yoga* on immunological paw edema in triple antigen sensitized rats

Groups	Percentage increase in paw volume	
	24 h	48 h
Control	39.43 ± 07.20	48.50 ± 7.11
Vachadhatryadi Avaleha	34.75 ± 14.65	34.16 ± 9.76
Adjuvant yoga	40.50 ± 09.12	46.79 ± 7.44

The test formulations were administered daily by the oral route to groups of rats (*n*=6) at 900 mg/kg dose for seven days. The data are expressed as Mean ± SEM

different rates during fetal and early postnatal life.[18] 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 endow it with better capacity to get adapted to different kinds of adverse conditions.[19] 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 *Prakshepa*) *per se* have anti-body enhancing effect [P<0.001; 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%) [Table 2]. The possible stages for this can be accelerated processing of antigen by the macrophages, enhanced secretion of cytokines like IL-$_4$ and tissue growth factor-b (TGB-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 immunological 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*[32-34] and *Ghrita*[35,36] 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.

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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. 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.
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, sushtura Samhita, commentary by Sri Dalhanacharya 'Nibandhasangraha', chaukhambha Orientalia. chapter 27 and 30. 8th ed. Varanasi, India: Chikitsasthana; 2005.
6. Vahata, Astangasamgraha, commentary by Indu, 'sasilekha', Chowkhamba Sanskrit series office. Varanasi, India: Uttara 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,editor. 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

35. In: Vaidya KS, editor. Kollam: Gopalan Pillai; 1946.
9. Sri Bhavamishra, 'Bhavaprakasha Nighantu'. commentary by Chunekar KC. Varansi, India: Chaukhambha Bharati Academy; 2006. P.11,13,14,16,17,19,31,43,80,94,95,243,342,454.
10. Sharangadhara. 'Sharangadhara Samhita', commentaries by Aadhamalla *'Dipika'* and Kashiram *'Gudartha- Dipika'*. 4th ed. Choukhambha Orientalia. Varanasi, India: *Prathama Khanda*; 2000, chapter 8, *Shloka* 01-04.
11. Paget GE, Barnes JM. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editor. Pharmacometrics. Vol. 1. New York: Academic press New York; 1969. p. 161.
12. Puri A, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbortristis* L. J Ethenopharmacol 1994;42:31-7.
13. Khan T, Tatke P, Gabhe SY. Immunological studies on the aerial roots of the Indian Banyan. Indian J Pharm Sci 2008;70:287-91.
14. Raghuramulu N, Nair KM, Kalyanasundaram S. A Manual of Laboratory Techniques. Hyderabad, India: National Institute of Nutrition (NIN); 1983. p. 246-53.
15. Bhattacharya. Manual Pre-conference, Annual conference of Indian Pharmacological society. Workshop on Research Methodology in pharmacology. 1993.
16. Bhatt KR, Mehta RK, Srivastava PN. A simple method to assess anti-inflammatory activity. Ind J Physiol Pharmac 1977;21:399.
17. Snedecor GW and Cochran WG. Statistical methods. 6th ed. Ames, Iowa: Iowa State University Press; 1967. p. 258-96.
18. Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell 1997;91:661-77.
19. Patwardhan B, Kalbag D, Patki PS, Nagasampagi BA. Search of Immunomodulatory Agents - a review. Indian Drugs 1991;28:249-54.
20. Scanlon VC, Sanders T. Essentials of anatomy and physiology. 5th ed. Philadelphia: F A Davis Company; 2003. p. 319-38.
21. Vohra SB. Antibacterial, antipyretic, analgesic, and anti-inflammatory studies on Acorus calamus Linn. Ann Natl Acad Med Sci 1989;25:13-20.
22. Dhir H, Agarwal K, Sharma A, and Talukder G. Modifying role of *Phyllanthus emblica* and ascorbic acid against nickel clastogenicity in mice. Cancer Lett 1991;59;9-18.
23. Suresh K, Vasudevan DM. Augmentation of murine natural killer cell and antibody dependent cellular cytotoxicity activities by *Phyllanthus emblica*, a new immunomodulator. J Ethnopharmacology 1994;44:55-60.
24. Tewari A, Sen SP, Guru LV. The effect of *Amalaki (Phyllanthus emblica)* Rasayana on biologic system. J Res Ind Med 1968;2:189.
25. Sharma RC, Gupta SK, Khurana SC, Arora RB. Some Parmacological properties of *Cyperus scariosus*. Indian J Physiol Pharmacol 1970;14:38.
26. Singh N, Kulshreshtha VK, Gupta HB, Bhargava KP. Pharmacological studies on *Cyperus rotundus*. Indian J Pharmacol 1969;1:19.
27. Anonymus. Pharmacological Investigations of certain medicinal plants and compound formulations used in Ayurveda and Siddha. New Delhi: CCRAS; 1996. p. 282.
28. Dahanukar SA, Karandikar SM, Desai M. Efficacy of *Piper longum* on childhood asthama. Indian Drugs 1984;21:384-8.
29. Rege NN, Thatte UM, Dahanukar S. Adaptogenic properties of six Rasayana herbs used in Ayurvedic medicine. Phytotherapy Res 1999;13:275-91.
30. Ali BH, Blunden G, Tanira MO, Nemmar MO. Some phytochemical, pharmacological and toxicological properties of ginger (*zingiber officinale roscoe*): A review of recent research. Food Chem Toxicol 2008;46:409-20.
31. Sunila ES, Kuttan G. Immunomodulatory and anti-tumour activity of *piper longum* Linn. and Piperine. J Ethnopharmacol 2004;90:339-46.
32. Kassim M, Mansor M, Achoui M, Yan OS, Devi S, Yusoff KM. Honey as an immunomodulator during sepsis in animal model. Sepsis 2009;13:16.
33. Marcucci MC. Propolis; chemical composition, biological properties and therapeutical utility. Apidologie 1995;26:83-9.
34. Viuda Martos M, Ruiz-Navajas Y, Fern´Andez-L´Opez J, P´Erez-´Alvarez JA. Functional Properties of Honey, Propolis and Royal Jelly. J Food Sci 2008;73:116-24.
35. Shriram Sheshgir Savrikar. 'Comparative Study of Physico-chemical characteristics of *'Accha Sneha'* and *'Siddha Sneha'* with reference to *'Guduchi Ghrita'*, the butter fat, medicated with *Tinospora cordifolia* (Willd.) Miers. PhD thesis submitted to Nanded, Maharashtra India: Swami Ramanand Teerth Marathwada University; 2006.
36. Ashok BK. "A detailed investigation on the gender and formulation influences on the expression of pharmacological activity in *Guduchi* (*Tinospora cordifolia* (Willd.) Miers.)". PhD thesis submitted to Jamnagar, Gujarat, India: Gujarat Ayurved University; 2009.

हिन्दी सारांश

इम्यूनोमोडुलेटरी एक्टीविटी ऑफ वचाधात्र्यादि अवलेह इन ऐल्बिनो रेट्स

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

प्रस्तुत शोधकार्य में वचाधात्र्यादि अवलेह की व्याधिक्षमत्व पर प्रभाव का प्रायोगिक अध्ययन ऐल्बिनो जाति के चूहों पर किया गया। इस अध्ययन में वचाधात्र्यादि अवलेह का हुयोमरल एंटीबोडी फोरमेशन एवं सेल मीडियेटिड इम्यूनिटी पर प्रभाव का मापन किया गया। शास्त्रीय विधि से निर्मित वचाधात्र्यादि अवलेह ९०० मि.ग्रा. प्रति किलोग्राम शरीरभार की मात्रा में ग्रुप बी के चूहों को खिलाया गया तथा इसके प्रभाव का (हिमएग्लुटीनेशन टाइटर) रक्तस्तम्भक क्षमता, पोंड्रल परिवर्तन(शरीर मापमें परिवर्तन), प्लीहा एवं लसिका ग्रन्थि की कोश रचना में परिवर्तन, केरेजीनिन द्वारा उत्पन्न पंजे के शोथ पर प्रभाव का अध्ययन किया गया। वचाधात्र्यादि अवलेह का शरीर में एंटीबोडी निर्माण को बढानेमें अत्यन्त प्रभावी रहा व इम्यूनोलोजीकल एडिमा को घटाने में मध्यम रूप से प्रभावी रहा। इस अध्ययन के निष्कर्षानुसार वचाधात्र्यादि अवलेह में व्याधिक्षमत्व बढाने की क्षमता है।