

Evaluation of immunomodulatory activity of “*Shirishavaleha*” –An Ayurvedic compound formulation in albino rats

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ABSTRACT

The immunomodulatory activity of *Shirishavaleha* prepared from two different parts of *Shirisha* (*Albizia lebbbeck* Benth), i.e., *Twak* (Bark) and *Sara* (Heartwood) as main ingredients was evaluated for humoral antibody formation and cell-mediated immunity in established experimental models. The study used Wistar rats of either sex weighing 200 ± 40 g, while the test drug was administered orally at a dose of 1.8 g/kg. Hemagglutination titer and body weight were recorded to assess effects on humoral immunity; immunological paw edema was assessed for cell-mediated immunity. *Shirishavaleha* prepared from heartwood shows significant enhancement in antibody formation, attenuation of body weight changes, and suppression of immunological paw edema, while *Shirishavaleha* prepared from bark shows weak immunomodulatory activity. The study therefore concludes that *Shirishavaleha* prepared from heartwood has significant immunomodulatory activity.

Key words: *Albizia lebbbeck*, *avaleha*, hemagglutination titer, immunomodulatory activity

INTRODUCTION

Traditional and folklore medicines continue to play important roles in health services around the globe.^[1] *Ayurveda*, the traditional medical system of India, describes thousands of herbal preparations. One, “*Shirisharishta*”, a well-known, fermented preparation of *Albizia lebbbeck* Benth., is a combination of 12 ingredients, with jaggery as its base, and used for the treatment of various disorders^[2]. The decoction of *Shirisha* bark has a protective action against bronchial asthma.^[3] Recent studies found the

plant to possess immunomodulatory^[4] and antihistaminic activity^[5]. Its extract has also been proven to be efficacious in cases of allergic rhinitis.^[6] Studies on other ingredients of the formulation taken individually, have also found immunomodulatory activity: *Pippali* (*Piper longum* Linn.),^[7] *Haridra* (*Curcuma longa* Linn.),^[8] *Kusta* (*Saussurea lappa* C. B. Clarke)^[9] *Shunthi* (*Zingiber officinale* Roscoe.),^[10] and *Ela* (*Elettaria cardamomum* Maton.)^[11].

However, being fermented, “*Shirisharishta*” may be considered inconvenient in a few ways: prolonged duration of preparation; decreased palatability for various age groups, etc. For this reason, we converted the fermented form (*Arishtha*) to a confection (*Avaleha*) as instructed according to the classical texts^[12]. Antitussive activity of the latter was evaluated.^[13]

The aim of the current study was to evaluate the immunomodulatory activity of two forms of “*Shirishavaleha*,” viz., one obtained from the bark (SAB) and the other from the heartwood (SAH).

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MATERIALS AND METHODS

Test formulations

Raw materials [Table 1] for the test formulation were procured from the pharmacy at the Institute for

Table 1: Formulation composition of Shirishavaleha

Ingredient	Botanical name	Part	Quantity		Amount taken in the current study
			Classical	Conversion	
<i>Kwatha Dravya</i>					
<i>Shirisha</i>	<i>Albizia lebbbeck</i> Benth.	Bark / Heartwood	1/2 Tula	2 400 g	1 250 g
<i>Jala</i> (w/w)	Potable water	-	2 Drona	24 566 ml	12 500 ml
	Reduced to				3 120 ml
<i>Praksepa Dravyas</i>					
<i>Pippali</i>	<i>Piper longum</i> Linn.	Fruit	1 Pala	48 g	24 g
<i>Priyangu</i>	<i>Callicarpa macrophylla</i> Vahl.	Flower	1 Pala	48 g	24 g
<i>Kushtha</i>	<i>Saussurea lappa</i> C. B. Clarke	Root	1 Pala	48 g	24 g
<i>Ela</i>	<i>Elettaria cardamomum</i> Maton.	Seed	1 Pala	48 g	24 g
<i>Nilini</i>	<i>Indigofera tinctoria</i> Linn.	Root	1 Pala	48 g	24 g
<i>Haridra</i>	<i>Curcuma longa</i> Linn.	Rhizome.	1 Pala	48 g	24 g
<i>Daruharidra</i>	<i>Berberis aristata</i> DC.	Stem	1 Pala	48 g	24 g
<i>Shunthi</i>	<i>Zingiber officinale</i> Roscoe.	Rhizome.	1 Pala	48 g	24 g
<i>Naga kesara</i>	<i>Mesua ferrea</i> Linn.	Stamen	1 Pala	48 g	24 g
<i>Madhura Dravya</i>					
<i>Guda</i>	Jaggery	-	2 Tula	9 600 g	5 000 g

(Metric equivalents for the classical weights are based on Ayurvedic Formulary of India Part - I, Second Revised Edition: Appendix V: Page 483)

Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurveda University, after authentication by the pharmacognosist. Two samples of *Shirishavaleha* were prepared from these materials, viz. one using *Shirisha* bark as the main ingredient and another using heartwood as the main ingredient. The *Avaleha* formulation was prepared by following classical guidelines^[12].

Animals

The study employed Wistar rats of either sex weighing 200 ± 40 g, obtained from the animal house attached to the Institute's pharmacology laboratory. They were housed in spacious polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries, and tap water given *ad libitum*. The animals were acclimatized for at least one week in laboratory conditions before the commencement of experiment in standard laboratory conditions, 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and 40 to 60% humidity. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (IAEC 09-10/05MD 07), and care of the animals was conducted according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals.

Dose fixation

Doses of the test formulations (both SAB and SAH) were calculated by extrapolating the human dose to animals (1.8 g/kg), based on the body surface area ratio referring to the standard table of Paget and Barnes (1969).^[14] The test formulation was suspended in distilled water (180 mg/ml) and administered orally at a volume of 0.5 ml/100 g body weight with the help of gastric catheter of suitable size sleeved on to a syringe nozzle to overnight fasted animals.

Effect on humoral antibody formation

The effect of the test drugs on antibody formation against sheep red blood cells (SRBC) was measured as described by Doherty.^[15] The selected animals were divided into four groups of six. The first group received distilled water, and served as the control group; the second group also received distilled water and served as SRBC control group. Calculated doses of the two samples of *Shirishavaleha* (SAB and SAH) were administered for 10 consecutive days to the third and fourth groups, respectively. On the third day, sheep blood was collected from the 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 was 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 of second to fourth groups. On the 11th day, the animals were weighed again and under light ether anesthesia, blood was drawn by puncturing supraorbital plexus and collected in plain tubes. Serum was separated from it and complements were inactivated by incubating it for 30 minutes at 56°C temperature in a serological water bath.

Estimation of antibody titer^[16]

The microtiter plate was filled with 0.1 ml sterile normal saline, and up to 16 serial two-fold dilutions of 0.1 ml serum in sterile saline solution were made in the microtiter plate. 0.1 ml of thrice saline-washed 3% SRBC was added to each well of the tray. Blood from the same animal (sheep)

Table 2: Effect on body weight

Treatment	Initial body weight (g)	Final body weight (g)	Change in body weight (g)	Percentage change
Control	218.00 ± 09.46	233.00 ± 10.02	14.67 ± 03.04	--
SRBC control	216.30 ± 05.42	185.00 ± 16.46	-31.33 ± 12.72 ^{##}	141.00 ↓
SAB	203.60 ± 09.73	215.33 ± 12.37	11.67 ± 02.98 ^{**}	137.25 ↑
SAH	216.33 ± 12.98	234.00 ± 14.09	17.67 ± 02.89 ^{**}	156.40 ↑

Data: Mean ± SEM ↑ - Increase ↓ - Decrease ^{##}*P*<0.01 compared with normal control group, ^{**}*P*<0.01 compared with SRBC control group
SRBC - sheep red blood cells; SAB - *Shirishavaleha* prepared from the bark; SAH - *Shirishavaleha* prepared from the heartwood

Table 3: Effect on antibody formation

Treatment	Antibody titer (Log ₂ values)	Percentage change
SRBC control	04.50 ± 00.24	--
SAB	04.85 ± 00.25	07.78 ↑
SAH	05.08 ± 00.13 [*]	12.99 ↑

Data: Mean ± SEM, ^{*}*P*<0.05 compared with SRBC control group. ↑ - Increase, SRBC - sheep red blood cells; SAB - *Shirishavaleha* prepared from the bark; SAH - *Shirishavaleha* prepared from the heartwood

Table 4: Effect on immunological paw edema

Treatment	Percentage increase in paw volume			
	24 hours	% inhibition	48 hours	Inhibition
Control	47.87 ± 9.68	-	38.90 ± 10.12	-
SAB	29.15 ± 05.37	39.10 ↓	18.25 ± 06.62	53.08 ↓
SAH	23.70 ± 02.15 [#]	50.50 ↓	11.16 ± 02.60 [*]	71.31 ↓

Data: Mean ± SEM ↓ - Decrease ^{*}*P*<0.05 compared with control group, SAB - *Shirishavaleha* prepared from the bark; SAH - *Shirishavaleha* prepared from the heartwood

was used for both sensitization and to determine antibody titer. The trays were covered and placed in a refrigerator overnight. Antibody titer (hemagglutination titer) was noted the next day. The titer was converted to log₂ values for easy between groups comparison.

Effect on cell-mediated immunity

Effect on cell-mediated immunity was evaluated using the procedure of Bhattacharya.^[17] The selected animals were divided into three groups of six animals in each group. First group received distilled water and served as normal control group. To the second and third groups, calculated doses of SAB and SAH were administered. All the animals were sensitized subcutaneously (0.5 ml/100 g body weight) on the first day of drug administration using the following solution: triple antigen (DPT) - 1 ml, normal saline (0.9%) - 4 ml, and potash alum (10%) - 1 ml. The solution pH was maintained between 5.6 and 6.8 using 10% sodium carbonate. The drug was administered for seven consecutive days. On the seventh day, one hour after drug administration, the initial volume of the left hind paw was noted, and 0.1 ml of the above solution was injected into plantar aponeurosis of same paw. The volume of immunological edema thus produced was measured by the volume displacement method^[18] 24 and 48 hours after the injection, using a plethysmograph. From this the percentage increase in paw volume, the index of edema formation, was calculated.

Statistical analysis

Results were presented as Mean ± SEM, statistical

significance of between groups difference was determined using unpaired Student's 't' tests.^[19]

RESULTS

A normal range of body weight increase was observed in control group rats. In contrast, an apparent decrease in body weight was observed in SRBC control rats. Pretreatment with SAB and SAH significantly attenuated body weight changes compared to the SRBC control group [Table 2].

Pretreatment with SAH significantly (*P*<0.05) enhanced antibody titer [Table 3] in SRBC presensitized rats in comparison with the SRBC control group. Treatment with SAB also enhanced antibody titer; however, the observed increase did not reach statistical significance.

In the SAB group, a decrease in paw volume was observed for both the 24- and 48-hour recordings, but did not reach statistical significance. In the SAH group, a statistically significant decrease in immunological edema was observed both after 24- and 48-hours [Table 4].

DISCUSSION

Gain in body weight indicates normal progressive health status of an organism while decrease is indicative of

degenerative changes in the body or certain organs. In the present study, presensitization of animals with SRBC led to significant decreases in body weight in 10 days. Both SAB and SAH formulations significantly attenuated SRBC-induced body weight changes. The observed effects may be attributed to prevention of SRBC- (antigen) induced degenerative changes in the body.

Hemagglutination antibody titer is a primary parameter for studying the humoral response. Antigen and antibody reaction results in agglutination. Antibody molecules secreted by plasma cells mediate the humoral immune response. SAH showed significant increase in hemagglutination titer, indicating immunostimulant activity. Such stimulation of humoral response to SRBC indicates enhanced responsiveness of macrophages, T and B lymphocyte subsets, involved in antibody synthesis.^[20] SRBC may therefore stimulate lymphocyte proliferation, in turn leading to production of cytokines activating other immune cells such as B cells.

Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). The Th-1 T-lymphocyte pathway controls cell-mediated immunity.^[21] Results show that SAH pretreatment significantly suppresses immunological edema suggesting that it may suppress that pathway. At the same time it seems to influence the Th-2 pathway, as indicated by enhanced antibody formation. Such effects could be produced by promoting formation and release of IL-4, while at the same time, decreasing formation of IL-12, the cytokine favoring Th-1 mediated immune response. Exact mechanism can only be elucidated by specific studies measuring different types of cytokines both *in vivo* and *in vitro*.

Immunomodulatory activity of the compound formulation may be understood using the concept of *Rasayana*. This indicates that components of the formulation help promote nutrition at *Rasa*, *Agni*, and *Srotas* levels, by the virtue of which immunomodulatory effects become possible.

In earlier research, *Visha* is understood as free radicals or antigens. The *Prabhava* of *Shirisha*, the main component of the formulation, is *Vishabara* (antagonistic to *Visha*). Other ingredients also possess the *Vishabara* property eg *Naga kesara*, *Haridra*, and *Nilini*. By the virtue of this, immunomodulatory activity could probably be anticipated. The components, *Pippali*, *Sunthi*, *Priyangu*, *Kusta*, *Daru haridra*, and *Naga kesara*, possess the properties *Dipana* and *Pachana*, which are known to promote digestion and revitalize metabolic activity resulting in improved nutritional status at the *Dhatu* level, and preventing further formation of *Ama* in the body. *Guda* (jaggery), a major ingredient of the formulation, contains bulk quantities of carbohydrates and other minerals, which, when

administered, directly enter into the pool of nutrition. This in turn helps improve tissue nourishment leading to subsequent *Rasayana* effects. These reasons may help explain why, in the presence of jaggery, the polyherbal combination possesses immunomodulatory activity.

CONCLUSION

From the present study, it is observed that both the test formulations possess immunomodulatory activity, that of SAH being more significant. Overall results suggest that both parts of *Shirisha* (bark and heartwood) can be used in the formulation. Indeed, since bark collection does not involve destructive collection practices, it should generally be used. However, where heartwood is abundantly available, it may be preferred. Even then a mixture of both would be useful.

REFERENCES

1. Premanathan M, Rajendran S, Ramanathan T, Kathiresan K, Nakashima H, Yamamoto N. A Survey of some Indian medicinal plants for anti-human immunodeficiency virus (HIV) Activity. *Indian J Med Res* 2000;112:73-7.
2. Shastri Ambika Datta, Bhaishajya Ratnavali, 15th ed. Varanasi, India: Chaukhambha Sanskrit Sansthan; 2002;72/72-74:765.
3. Bhattathri PP, Rao PV, Acharya MV, Bhikshapathi T, Swami GK. Clinical Evaluation of Shirisha Twak Kwatha in the management of Tamaka Shwasa. *J Res Ayurveda Siddha* 1997;18:21-7.
4. Barua CC, Gupta PP, Patnaik GK, Misra-Bhattacharya S, Goel RK, Kulshrestha DK, *et al.* Immunomodulatory Effect of Albizzia lebeck. *Pharm Biol* 2000;38:161-6.
5. Tripathi RM, Das PK. Studies on anti-asthmatic and anti-anaphylactic activity of Albizzia lebeck. *Indian J Pharm* 1977;9:189-94.
6. Pratibha N, Saxena VS, Amit A, D'Souza P, Bagchi M, Bagchi D. Anti-inflammatory activities of Aller-7. A novel polyherbal formulation for allergic rhinitis. *Int J Tissue React* 2004;26:43-51.
7. Sunila ES, Kuttan G. Immunomodulatory and Antitumor activity of Piper longum Linn and Piperine. *J Ethnopharmacol* 2004;90:339-46.
8. Yadav VS, Mishra KP, Singh DP, Mehrotra S, Singh VK. Immunomodulatory Effects of Curcumin. *Immunopharmacol Immunotoxicol* 2005;27:485-97.
9. Fulzele SV, Bhurchandi PM, Kanoje VM, Joshi SB, Dorle AK. Immunostimulant activity of Ashtamangala Ghrita in rats. *Indian J Pharmacol* 2002;34:194-7.
10. Liu H, Zhu Y. Effect of alcohol extract of *Zingiber officinale* rose on immunologic function of mice with tumor. *Wei Sheng Yan Jiu* 2002;31:208-9.
11. Majdalawieh A, Carr R. In vitro investigation of the potential immunomodulatory and anti-cancer activities of black pepper (Piper nigrum) and cardamom (Elettaria cardamomum). *J Med Food* 2010;13:371-81.
12. Sharangadhara, 'Sharangadhara Samhita', commentaries by Aadhamalla 'Dipika' and Kashiram 'Gudarth- Dipika'. 4th ed. Shloka 01-04. Prathama Khanda. Varanasi, India: Choukhambha Orientalia; 2000.
13. Singh YS, Galib, Ashok BK, Prajapati PK, Ravishankar B. Evaluation of Anti Tussive activity of Shirishavaleha - An Ayurvedic Compound Formulation in Sulphur Dioxide Induced

- Cough in Mice. Indian Drugs 2010;47:38-41.
14. Paget GE, Barnes JM. Evaluation of drug activities, pharmacometrics. In: Lawranle DR, Bacharch AL, editors. New York: Academic press; 1969;1:161.
 15. Doherty NS. Selective effect of immunosuppressive agents against delayed hypersensitive response and humoral response to sheep red blood cell in mice. Agents Actions 1981;11:237-42.
 16. Furine MJ, Norman PS, Creticos PS. Immunotherapy decreases antigen induced eosinophil cell migration in to the nasal cavity. J Allergy Clin Immunol 1991;88:27-32.
 17. Bhattacharya. Manual Pre-conference, Annual conference of Indian Pharmacological society. Workshop Res Methodol Pharmacol 1993.
 18. Bhatt KR, Mehta RK, Srivastava PN. A simple method for recording antiinflammatory effects on rat paw oedema. Indian J Physiol Pharmacol 1977;21:399-400.
 19. Snedecor GW, Cochran WG. Statistical methods. 6th ed. Ames, Iowa: Iowa State University Press; 1967. p. 258-96.
 20. Benacerraf B. A hypothesis to relate the specificity of T lymphocytes and the activity of I region specific Ir genes in macrophages and B lymphocytes. J Immunol 1978;120:1809-12.
 21. Lele RD. Ayurveda and modern medicine. 2nd ed. Mumbai, India: Bharatiya Vidya Bhavan; 2001. p. 475.

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