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Website: www.ayujournal.org
DOI: 10.4103/0974-8520.82040
Quick Response Code:

Pharmacological Research

Evaluation of immunomodulatory effect of *Ranahamsa Rasayanaya* – A Sri Lankan classical *rasayana* drug, on experimental animals

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Abstract

Immunity plays a key role in maintaining the health of an individual. Therefore, the rational modulation of the immunity through psycho-neuro-endocrine-immune (PNI) axis is useful for the prevention as well as for the curing of the diseases. As immunomodulation is a parameter for evaluation of the *rasayana* effect of a drug, the same has been studied to assess the *rasayana* effect of *Ranahamsa Rasayanaya* (RR). Experimental models such as antibody formation against sheep red blood cells (SRBC) and cell mediated immunity (CMI) have been carried out befitting on Wistar strain albino rats to determine the immunomodulatory effect plus *rasayana* effect of RR. Statistically significant increase in body weight, nonsignificant increase in antibody formation against SRBC, highly significant decrease in CMI were observed in the treatment groups, when compared to the standard control group. These results show the probable immunomodulatory and anabolic activities of the test drug. Outcome of these studies validate the strong *rasayana* effect of the test drug claimed by the traditional practitioners of Sri Lanka.

Key words: *Ranahamsa Rasayanaya,* immunomodulation, Sheep RBC, cell mediated immunity, psychoneuroimmunology, anabolic activity

Introduction

Rasayana therapy is indicated for the prevention as well as for the curing [1,2] of the diseases, in the science of Ayurveda. Immunomodulation may be considered as a major mechanism contributing to these effects of *rasayana* therapy. *Ranahamsa Rasayanaya*[3] (RR), a renowned *rasayana* formulation found in Sri Lanka, used in chronic and severe debility of the body secondary to major illnesses including cachexia associated with tuberculosis, diabetes mellitus, etc.[3,4] It is the need of the hour to verify such claims by using the modern scientific parameters, thereby establishing the indigenous knowledge on a par with the todays development. Bioassays for pharmacological screening is recommended along with other parameters by World Health Organization (WHO) in 1992, for the authentication and standardization of plant drugs. Experimental models such as antibody formation against sheep red blood cells (SRBC) and cell mediated immunity (CMI) are considered as two standard bioassays for pharmacological screening of immunomodulation.

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Aims and Objectives

1. Evaluation of the test drug's effect on humoral immunity and
2. Determination of test drug's effect on CMI.

Plan of study: This pharmacology study has also been carefully planned according to the current scientific and ethical guidelines to evaluate the key objectives on immunomodulation, and accordingly the experiments were carried out. The following pharmacological studies were approved by the Institutional animal ethics committee (IAEC).

Materials and Methods

Procurement of test drug

The test formulation, *Ranahamsa Rasayanaya* of two different batches, was bought from Sri Lanka Ayurvedic Drugs Corporation.

Animals

1. Wistar strain albino rats of either sex weighing between 180 g and 230 g were used for experimental study.
2. The animals were obtained from the animal house attached to the pharmacology laboratory of Institute for Post Graduate Teaching and Research in Ayurveda,Jamnagar.
3. Animals were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature (22 ± 2ºC) and humidity (50–60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given *ad libitum*.
4. All the experiments were carried out after obtaining permission from "Institutional animal ethical committee" (IAEC).

Dose fixation and schedule

The dose calculation was done on the basis of body surface area ratio using the table of Paget and Barnes[5] and a dose of 900 mg/kg/day from both the batches of the test drug and the vehicle were administered according to the body weight of the individual animals orally to the respective groups, with the help of a gastric catheter of suitable size sleeved to a syringe nozzle. The test drug was administered from a fresh stock solution prepared by mixing the test formulation with tap water to a suitable concentration.

Statistical analysis: Evaluation of the data through statistical estimations within the group and comparison between the groups after treatment (AT) were assessed by using paired and unpaired Student's *t* test respectively. Being number of observations (*n*) in each group below 30, Student's *t* test was employed for the purpose. The statistical estimations particularly sample mean, standard deviation (SD), standard error of mean (SEM), calculated *t* value and probability (*P*) value were obtained by applying the standard formulae. Probability (*P*) values of *t* are tabulated for various degrees of freedom (df) according to the number of observations. If the obtained *P* value is less than 0.05, then the result is considered as statistically significant.

Effect on humoral antibody formation

Wistar strain albino rats of either sex weighing between 210 g and 230 g were randomly grouped into three groups. Group A served as the control group and received tap water. Both the batches of the test drug were administered to both treatment groups (Groups B and C) for ten consecutive days. On the third day, 20% SRBC solution prepared according to the standard protocol was used to sensitize all the animals. 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 blood was collected from the sacrificed animals and the separated serum was incubated for 20 min at 55 - 56 °C temperature in a serological water bath to inactivate the complement system contained in the serum. The dissected spleen, thymus and lymph nodes were weighed and the weights were recorded for individual animals with respect to the groups. Tissues were preserved with 10% formaldehyde solution for fixation and for histological studies. For the estimation of antibody formation, the microtiter 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 microtiter plate. A measured quantity of 0.1 ml of thrice saline washed 3% SRBC was added to each well of the tray. Sheep blood 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 and the titer was converted to $\log_2$ values for easy comparison.

Procedure followed for preparation of histopathological slides

Fixation, tissue processing, section cutting and staining procedure with two reagents, i.e.: Mayer's Haematoxylin Stain and Eosin Stain had been done systematically according to the protocol given by Raghuramulu.[6] After that, the slides are mounted with diphenyl phthalein xylene (DPX) with a cover slip. The slides were viewed under Trinocular Research Carl Zeiss Microscope (Germany) at various magnifications for histological findings.

Effect on CMI

Wistar strain albino rats weighing from 180 g to 200 g were used and they were randomly assigned into three groups similar to the above experiment. All of them were sensitized subcutaneously on first day of drug administration by following solution.

Triple antigen (DPT)	- 1 ml
Potash alum (10%)	- 1 ml
Normal saline (NS)	- 4 ml

pH of the above reagent was maintained between 5.6 - 6.8 using 10% sodium carbonate solution. On seventh day the initial paw volume of left hind paw up to tibio-tarsal joint was measured by using plethysmograph and after that, 0.1 ml of above solution was injected into plantar aponeurosis of the same paw. Volume of immunological edema thus produced was measured by volume displacement method[7] after 24 h and 48 h. Percentage increase in paw volume, which is the index for edema formation over initial value, was calculated. Statistical comparison of the values of Group A with the values of Groups B and C and thereby CMI response was assessed.

Observations and Results

A. Effect on humoral antibody formation against SRBC

Table 1 data shows an apparent increase in the body weight in both the test drug treated groups. The observed weight gain is statistically significant.

Marginal decrease in thymus weight was found in the treatment groups and the results were statistically nonsignificant in both groups [Table 2].

The data of Table 3 reveals a moderate and statistically non-significant increase in spleen weight in sample II administered group.

Statistically nonsignificant increase in antibody formation was observed against SRBC pre-sensitization in both the treated groups in comparison to control group [Table 4].

Histopathological study

Thymus

Microscopic examination of thymus sections from both the drug treated groups did not show any change in the cytoarchitecture in comparison to thymus section from SRBC control rats [Figure 1].

Table 1: Effect of two different samples of test drug on body weight in SRBC pre-sensitized albino rats

Groups	Body weight (g)			
	Initial	Final	Actual change	% change
Control	210.00 ± 08.16	213.33 ± 06.67	03.33 ± 02.11	-
RR I	221.67 ± 06.54	258.33 ± 10.14	36.67 ± 06.15*	16.50 ↑
RR II	223.33 ± 10.22	245.00 ± 10.25	21.67 ± 04.77*	09.95 ↑

Data: Sample mean ± SEM, ↑ - Increase, * - $P<0.05$

Table 2: Effect of two different samples of test drug on thymus weight in SRBC pre-sensitized albino rats

Groups	Absolute weight (g)	Relative weight (g/100 g body Wt)	% change
Control	472.83 ± 31.12	185.13 ± 05.06	-
RR I	449.67 ± 14.85	174.90 ± 06.53	05.35 ↓
RR II	409.17 ± 25.68	167.31 ± 09.24	09.24 ↓

Data: Sample mean ± SEM, ↓ - Decrease

Table 3: Effect of two different samples of test drug on spleen weight in SRBC pre-sensitized albino rats

Groups	Absolute weight (g)	Relative weight (g/100 g body Wt)	% change
Control	563.50 ± 44.89	221.26 ± 12.80	-
RR I	567.17 ± 39.63	218.66 ± 09.85	-
RR II	590.67 ± 29.66	243.20 ± 15.51	11.81 ↑

Data: Sample mean ± SEM, ↑ - Increase

Table 4: Effect of two different samples of test drug on antibody formation against SRBC in SRBC pre-sensitized albino rats

Groups	Antibody titer (log_2 value)	% change
Control	3.928 ± 0.292	-
RR I	4.506 ± 0.155	18.621↑
RR II	4.506 ± 0.155	18.621↑

Data: Sample mean ± SEM, ↑ - Increase

Table 5: Effect of different samples of test drug on immunological paw edema in pre-sensitized albino rats

Group	24 h	% change	48 h	% change
Control	60.84 ± 03.169	-	61.75 ± 04.155	-
RR I	34.74 ± 03.362***	57.10 ↓	35.56 ± 02.352***	57.58 ↓
RR II	25.04 ± 02.142***	41.20 ↓	27.88 ± 02.504***	45.15 ↓

Data: Sample mean ± SEM, ↓ - Decrease, *** - $P<0.001$

Lymph nodes

Slightly decreased cellularity was observed in sections of lymph node obtained from both the test drug administered groups in comparison to sections from control group [Figure 2].

Spleen

Sections of spleen from the test drug administered groups showed normal cytoarchitecture in comparison to sections of spleen from control SRBC group [Figure 3].

B. Effect on CMI

In comparison to control group, reduction of immunological paw edema in both the treated groups is less and the observed difference is statistically highly significant at both 24 h and 48 h of post sensitization [Table 5].

Discussion

The obtained results show, the well responsiveness of the employed experimental models in the study. The analysis of the results and the outcomes are discussed below in the context of the aims and objectives of the present study.

Immunomodulatory activity

With immunomodulatory models, it was intended to study the effect of the test drug on the different faculties of body's immune system. Immunomodulation can be an important attribute in the test drug and strengthening of immunocompetence in a controlled manner would help in the disease management.

Effect of test drug on humoral antibody formation

The *in vivo* persistence of immunogenic material has been

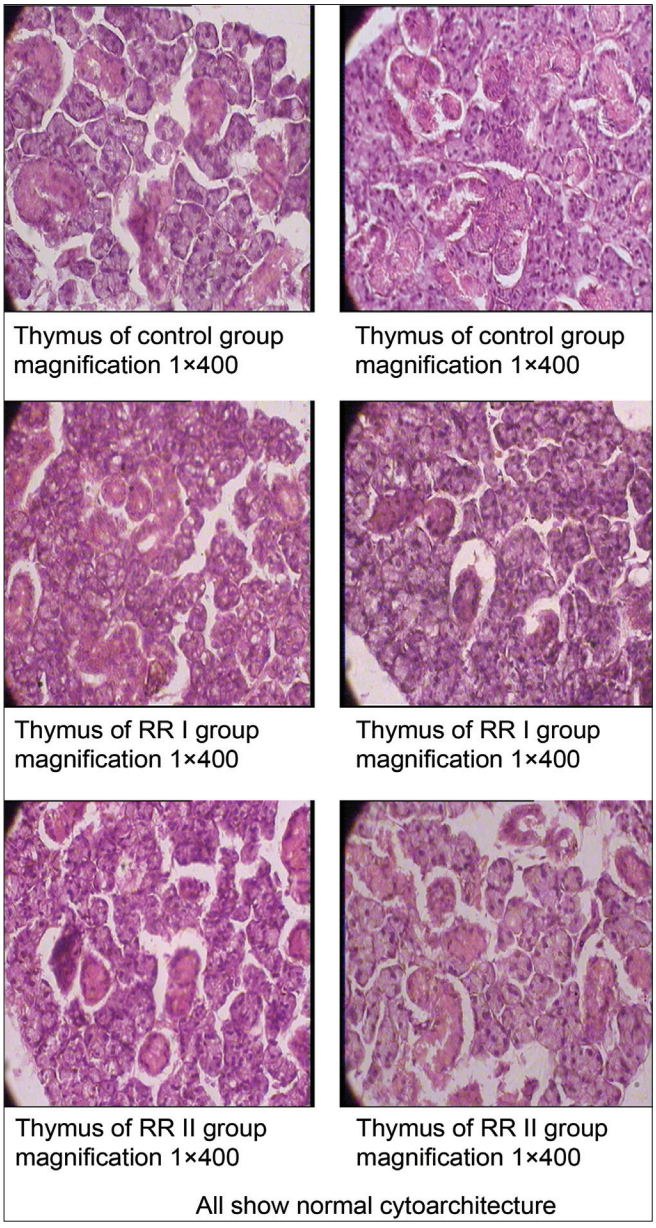

Figure 1: Evaluation of immunomodulatory effect of Ranahamsa Rasayanaya - A Sri Lankan classical rasayana drug on Thymus of experimental animals

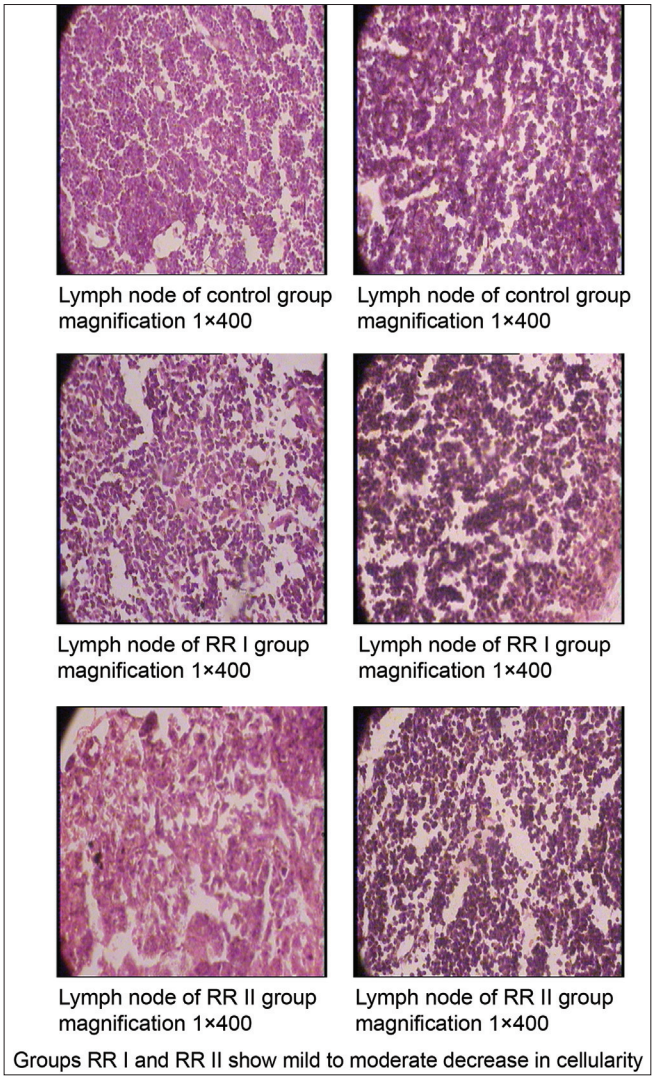

Figure 2: Evaluation of immunomodulatory effect of Ranahamsa Rasayanaya - A Sri Lankan classical rasayana drug on Lymph nodes of experimental animals

determined by the ability of organ extracts to sensitize non-immune or pre-sensitized recipients or by tracing labelled antigens.[8] The same is the basis for this experimental study. SRBC antigens was immunogenic only for 14 days *in vivo*.[8] Thus the experiment should be completed before 14 days. Here the treatment duration was 10 days, including the post-sensitization eight days. With 10 days treatment duration, both the samples did not influence the weight of the thymus and spleen in a significant manner. This shows that both the batches of the test drug do not produce any cytotoxic effects. The cytoarchitecture of thymus and spleen did not show any significant change in the test drug treated SRBC sensitized rats, but in the lymph node slight decrease in cellularity was observed, which points out the test drug could be a potential candidate for the treatment of lymphadenitis. Both batches of the test drug moderately stimulate the antibody formation against SRBC in comparison to control rats and magnitude was similar in the test drug from both batches. The overall indication is that, the test drug stimulated moderate antibody formation, but it failed to reach a statistical significant level. This may be possible due to two things:

1. caused by antigen-induced tolerance due to high dose of sheep RBC[9] or
2. caused by low dosage of the test drug.

If the latter is responsible for this, it may be overcome by the high dosage of the test drug. Both the batches of the test drug significantly increased the body weight in comparison to SRBC alone sensitized rats.

Effect of test drug on CMI

Two test drug samples suppressed CMI in a highly significant manner at both 24 h and 48 h post challenge of triple antigen.

The analysis of the data pertaining to the immunomodulation

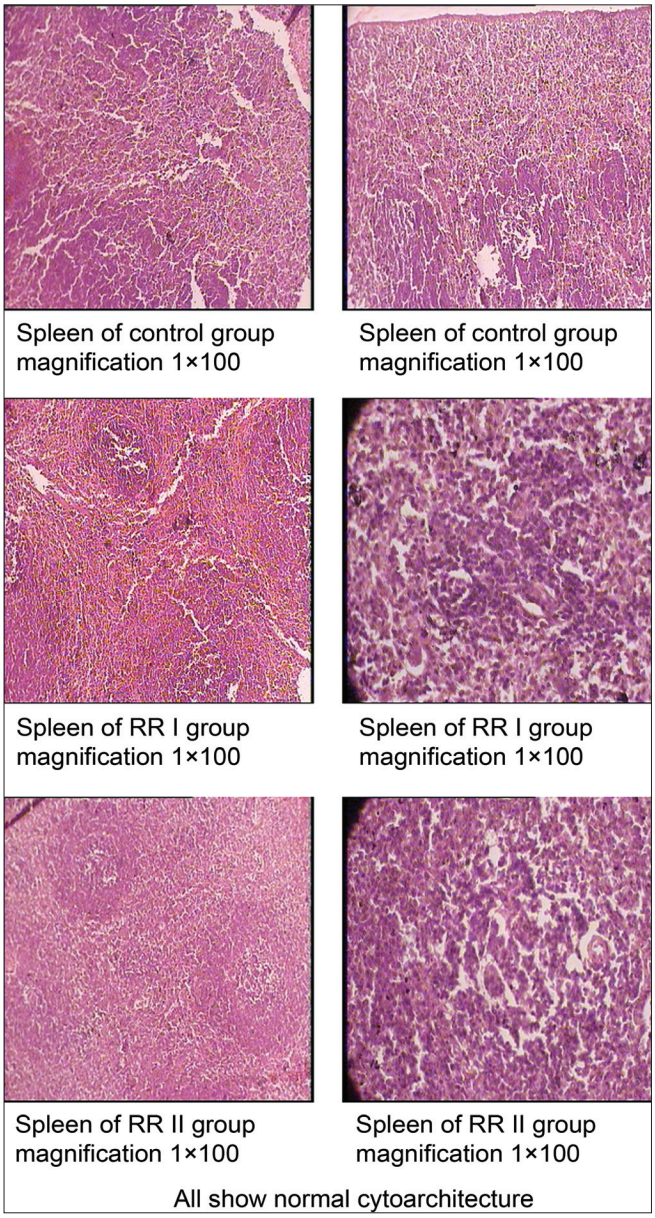

Figure 3: Evaluation of immunomodulatory effect of Ranahamsa Rasayanaya - A Sri Lankan classical rasayana drug on Spleen of experimental animals

study indicates that both the batches of the test drug are immunologically active and produce stimulation of antibody formation and suppress CMI. Since no cytotoxic effect is observed, it can be suggested that, this immunomodulation does not involve modification of cell turnover of cell involved in immunological mechanism. Enhanced antibody formation requires activation of B lymphocytes and their conversion to plasma cells, where T-lymphocytes play an important role.[10]

Conclusion

These experimental studies were carried out as a part of standardization of the test drug, especially regarding the immunomodulatory activity, anabolic activity, etc. The analysis of the results of these studies clearly indicates that, both the batches of the test formulation have powerful immunomodulation activity in the form of antibody formation enhancing and CMI suppressing effects. This may be an example for a regulated inflammation by the acquired immune response. By these findings, it could well be hypothesized that the test drug may have altered the body's psychoneuroimmunology (PNI) axis. Further they also possess strong anabolic activity. Histological studies on lymph nodes revealed mild to moderate decrease in cellularity in both the treatment groups, which indicate the potential therapeutic application of the test drug in the lymphadenitis. These all outcomes substantiate the definite *rasayana* effect of *Ranahamsa Rasayanaya*, claimed by the traditional practitioners of Sri Lanka.

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