AYUSHDHARA ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

TO STUDY IMMUNOMODULATORY EFFICACY OF *KARANJA* (*PONGAMIA PINNATA* PIERRE) SEED *CHURNA* IN SWISS ALBINO MICE

Gupta Chandni[1]*, Upadhayaya Ashwani[2]
*[1]Lecturer, [2]Professor & H.O.D., P.G. Dept. of Dravyaguna, R.G.G.P.G.AYU. College, Paprola, H.P., India.

KEYWORDS: *Karanja*, *Pongamia Pinnata,* SRBC, Cell Mediated Immunity, Sheep's blood.

***Address for correspondence**
Dr Gupta Chandni
Lecturer, P.G. Dept. of Dravyaguna, R.G.G.P.G.AYU. College, Paprola, H.P., India
Email: kaurachandni17@gmail.com
Mobile: 8679513399

ABSTRACT

Objectives: The present study was carried out to evaluate the immunomodulatory efficacy of *Karanja* (*Pongamia pinnata* Pierre) Seed *Churna* in aqueous solution. **Methods:** The animals for this trial were rats of either sex and blood that was used was sheep's blood. The study was conducted in three groups having 6 rats in each group. First group was control group and tap water was administered to this group orally, second and third group received the test drug aqueous solution at the dose 800mg and 4000mg/kg body weight respectively for 10 consecutive days. The test drug was evaluated for effect on humoral antibody formation, on cell mediated immunity and spleen and thymus weight gain. Histopathological studies were also performed on spleen and thymus in SRBC pre-sensitized rats. **Results:** The data on the effect of test drug on antibody formation against sheep red blood cells shows marginal statistically non-significant decrease in antibody titre at both lower and higher dose levels. Cell mediated immunity only at higher dose level in 48 hours reached statistically significant level whereas non-significant changes were observed in the weight of spleen and thymus. **Conclusion:** *Karanja* seed possess significant cell mediated immunity and non-significant effect on antibody formation and also non-significant effect on spleen and thymus weight gain.

INTRODUCTION

Pharmacology is the science of drugs. In a broad sense, it deals with interaction of exogenous administered chemical molecules (drugs) with the living systems.[1] The object of Pharmacology is to provide a scientific foundation for therapeutics and to increase the resources of the art of healing the exact way in which a drug changes the diseased condition can often be followed only imperfectly in man and hence, recourse has to made to experiments on healthy or diseased animals to elucidate the principles on which it should be employed. Knowledge of the mode of action of a drug obviously greatly enhances prediction from animal studies of what happened in man. Experimental study and the clinical study are the two aspects of the drug research. Experimental study can give us the better idea about the exact properties and action of the drugs. Moreover, there is a need to explain the drug activity in terms of current modern medical concepts by employing suitable experiments and methods so that the Ayurvedic means and methods are accepted globally. Further it also ensures scientific validation of Ayurvedic concepts and drugs. *Karanja* (*Pongamia pinnata* Pierre) is one of the well-known medicinal plants growing everywhere in India. It has been advocated in treating a broad range of diseases. The Ayurvedic treatises appreciated the therapeutic activities of this plant particularly in skin diseases.[2] In the present study an effort has been made to study the immunomodulatory properties of the *Karanja* in experimental animals. Immuno-modulation is modulation (regulatory adjustment) of the immune system. It has natural and human-induced forms, and thus the word can refer to the following: Homeostasis in the immune system, whereby the system self-regulates to adjust immune responses to adaptive rather than maladaptive levels (using regulatory T cells, cell signaling molecules, and so forth). Immunomodulation as part of immunotherapy, in which immune responses are induced, amplified, attenuated, or prevented according to therapeutic goals.[3]

Aims and objectives

To study the immunomodulatory activity of aqueous solution of *Karanja* seed *Churna* (powder) which includes the study of its effect on humoral antibody formation, effect on spleen and thymus weight and its effect on cell mediated immunity.

Materials and methods

Drug material

The drug *Pongamia pinnata* Pierre, seed powder (*Churna*) sample was supplied by Pharmacy, Gujarat Ayurveda University, Jamnagar and identified and authenticated in Pharmacognosy laboratory. For administering to the experimental animals, a drug suspension was made with requisite quantity of distilled water according to the dose required.

Animals

Swiss albino mice and Charles Foster strain albino rats were obtained from the animal house attached to the I.P.G.T.R.A, Gujrat Ayurveda University, Jamnagar. The animals were maintained on Navchakan Oil Mills, "Amrut" Brand rat pellets feed and tap water given *ad libitum.* The animals were maintained under normal ambient conditions. Each experimental group consisted 6 rats of either sex. Control group received equal quantity of the Vehicle (distilled water) used for the preparation of the test drug suspension.

Chemicals

Sheep blood was collected fresh from the local slaughter house in a sterile glass bottle containing autoclaved ACD solution.

Instruments used

Sterilizer, surgical instruments, cotton, syringes, needles, centrifuge, refrigerator, feeding syringes and tubes, serological water bath, vortex mixer, Alserver's solution, formaldehyde solution, haemotoxylin and eosin stain, haemagglutination titre tray, microtiter plates and picric acid.

Route of administration

The drug was administered by oral route with the help of a gastric catheter sleeved on to a syringe. The dose of the drug was calculated by extrapolating the human dose to animals based on the body surface area ratio by referring to the standard table of Paget and Barnes (1994). [4]

Statistical analysis

Student's 't' test for unpaired data has been used for analyzing the data generated during the study.[5]

Immunomodulation Activity

Effect on Humoral Antibody Formation

Rats of either sex in the body weight ranges between 160-254 g were used in the present study. Three groups each with six rats were included. First group was kept as control and tap water was administered by oral route. Second group was kept as a high dose group, received *Karanja* seed in a dose of 800 mg/ kg body weight daily for 10 consecutive days, while group third was kept as a low dose group and was administered *Karanja* seed in a dose of 400 mg per kg body weight in aqueous solution. On the third day of test drug administration sensitizing agent was injected intra-peritoneally. Sensitizing agent used was sheep red blood corpuscles (30% v/v) in the dose of 10 ml per kg body weight. Sheep blood was collected aseptically from the city slaughter house in a sterilized bottle containing ACD solution. SRBC were thoroughly washed with sterile normal saline and stored in Alserver's solution in a refrigerator till experimentation. Blood from sterile animal was used both for sensitizing and to determine antibody titre. On the eleventh day, rats were sacrificed by cervical dislocation and blood was collected. Serum was separated from it and complement in it was inactivated by heating at 56 degree Celsius for 30 minutes in serological water bath. Serial two folds dilutions of the serum in sterile saline solution were made in the volume of 0.1 ml in micro-titreplate. 0.1 ml of thrice saline washed 2% SRBC was added to each well. The trays were covered and placed in a refrigerator overnight. Haemagglutination titre with SRBC were prepared (Fig. 1). Haemagglutination titre was noted and titres were converted to Log 2 values for easy comparison. Spleen and thymus were also dissected out immediately after the rats were sacrificed. After noting the weight the tissues were transferred to 10 % formaldehyde solution for fixation and later on processed for histological studies following standard procedures as described in NIN manual.[6] Microtome sections were cut, processed and stained with haemotoxylin and eosin. The sections thus obtained were scanned in a binocular research microscope under different magnifications. Changes if any in the cyto- architecture were noted down.

Effect of test drug on cell mediated immunity

Unlike antibody mediated immune response, which is mediated through the formation of antibody by the plasma cells, in cell-mediated immunity T-Lymphocyte directly react with antigen to cause its destruction. Cell mediated immunity is also mediated by release of lymphokines, antibodies and complement are not involved in these reactions. This phenomenon is responsible for the rejection of foreign cells. Organ transplantation is one such reaction.

The test drug was evaluated to assess its effect on cell- mediated immunity by noting its effect on immunological inflammation produced by pedal injection of a suspension of SRBC. SRBC was thoroughly washed with sterilized normal saline by centrifuging and stored in Alserver's solution in a refrigerator till experimentation. Sterile thrice washed 4% SRBC suspension was injected 0.5 ml per 100 g body weight to each rat on the first day of drug administration. The drug treatment was continued for six days. Injecting 0.05 ml of similar suspension in the right hind paw on the sixth day of sensitization induced immunological oedema. Volume of the oedema was measured by volume displacement method (Bhatt et al 1977)[7], before sensitization, 24 and 48 hours after the second injection of SRBC into hind paw. Percentage increase over initial value was calculated. The values from control group were compared to test drug administered group to ascertain whether the drug modulates cell- mediated immunity or not.

RESUTS AND OBSERVATIONS

(A) Effect on antibody formation

The data on the effect of test drug on antibody formation against sheep red blood cells (SRBC has been presented in table 1).

Table 1: Effect of *Karanja* seed *Churna* on antibody formation against SRBC in rats

Group	Dose mg/kg	Antibody titre (log_2 values)
Control	-	6.01±0.42
Karanja seed *Churna*	400	5.89±0.64
Karanja seed *Churna*	800	5.77±0.49

Data: mean ±S.E.M.

A marginal statistically non-significant decrease in antibody titre was observed at both lower and higher dose levels. Decrease was more in higher dose level in comparison to lower dose level.

A) Effect on spleen and thymus weight

The data on the effect of test drug on the weight of spleen and thymus has been presented in table 2

Table 2: effect of *Karanja* seed *Churna* on spleen and thymus in SRBC pre- sensitized rats

Group	Dose mg/kg	Spleen weight		Thymus weight	
		Absolute (g)	Relative (g/100g body weight)	Absolute (g)	Relative (g/100g body weight)
Control	-	0.324±0.15	0.174±0.007	0.506±0.042	0.269±0.002
Karanja seed *Churna*	400	0.348±0.020	0.198±0.015	0.543±0.06	0.278±0.003
Karanja seed *Churna*	800	0.388±0.24	0.198±0.0008	0.693±0.04	0.356±0.04

Data: mean ±S.E.M.

The values have been presented in both in terms of absolute as well as relative values. a apparent increase in spleen weight and thymus weight was observed both with respect to absolute and relative values in lower dose as well as higher dose level. However, none of the values in both the groups reached statistically significant levels. Increase in weight was more in higher dose level than in lower dose level, except in relative spleen weight, where the increase was the same in both the groups.

B) Effect on cell mediated immunity

The effect of *Karanja* seed *Churna* on cell mediated immunity was evaluated by noting the effect of its administration on 4% SRBC induced pedal oedema in pre-senstized rats. The data pertaining to the test is presented in table 3.

Table 3: Effect of *Karanja* seed *Churna* on 4% SRBC induced and hind paw oedema in pre sensitized rats

Group	Dose mg/kg	Percentage increase in paw volume	
		24 hours	48 hours
Control		28.77±4.03	7.31±0.81
Karanja seed *Churna* low dose	400	23.73±4.71	2.60±1.86
Karanja seed *Churna* high dose	800	14.73±6.42	1.85±1.85*

Data: mean ±S.E.M. *P<0.05

At the lower dose level, 17.51% decrease in paw oedema in 24 hours and 64.43% decrease in 48 hours was observed whereas at higher dose level, 48.80% decrease in 24 hours and 74.69% decrease in 48 hours was observed. However, only one value at higher dose level in 48 hours reached statistically significant level ($p<0.05$).

C) Histopathological Studies

The following observations were made on histopathological study of spleen and thymus in SRBC pre-sensitized rats.

a) Spleen

Photomicrographs of sections of spleen obtained from different groups are presented in Fig. 2a, 2b, 12c. In lower dose drug administered group 9 (Fig 1b), increase in proportion of white pulp was observed in comparison to spleen sections from control rats (Fig 2a), whereas no change could be observed at higher dose level (Fig2c).

b) Thymus

Fig. 3a, 3b and 3c depict photomicrographs of sections of thymus obtained from different groups. In the group receiving 400 mg/ kg dose of *Karanja* seed *Churna* (Fig. 3 b), moderate increase in cellularity and vacuolization were observed. In 800 mg/kg dose given group (Fig. 3c), increase in cellularity was observed.

DISCUSSION

The *Karanja* seed *Churna* did not produce significant decrease in the antibody titre against SRBC in rats. However, significant antagonism of immunological paw oedema was observed at higher dose level. Moderate increase in spleen weight and thymus weight was observed at higher dose level. In cell mediated immunity, foreign antigen is processed and presented to CD4helper T-cell which elaborate IL-2 and other cytokines that in turn stimulate proliferation and maturation of precursor cytotoxic lymphocytes, that have been activated by antigen presented with class-I major histo-compatibility complex. The mature CTL (killer cells) recognize cells carrying the antigen and lyse them. Another sub-populations of T-lymphocytes termed suppressor cells modulate the functioning of the other lymphocytes by elaborating tonic inhibitors. If their action is inhibited, it will lead to immuno-stimulation and if their functioning is enhanced, it may lead to immuno- suppression. It can be suggested that the test drug may have some modulatory effect on suppressor T-cells. As described above, there are many potential sites for drug action. It is possible that the test drug may contain active principles, which modulate immune response by acting at one of the above potential sites.

CONCLUSION

Results of the present study clearly show that *Karanja* (*Pongamia pinnata* Pierre) seed possess significant cell mediated immunity and non- significant decrease in antibody titre against SRBC in rats. The increase in weight of spleen and thymus was also non- significant. However, some increase in weight and antibody titre was observed at higher doses.

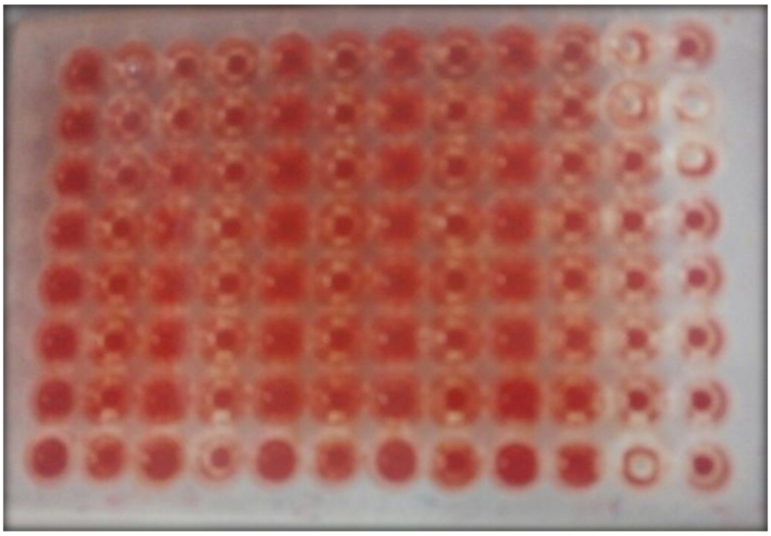

Fig. 1- Showing haemagglutination titre with SRBC

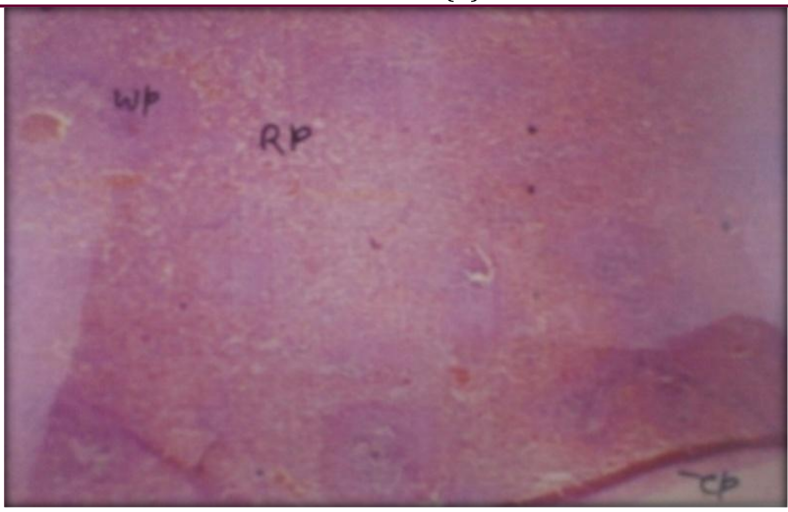

Fig. 2a: Photomicrograph of section of spleen from control SRBC sensitized rats (I X 32 magnification) Wp: white pulp Rp: red pulp Cp: capsule (Note : Slightly increased proportion of white pulp)

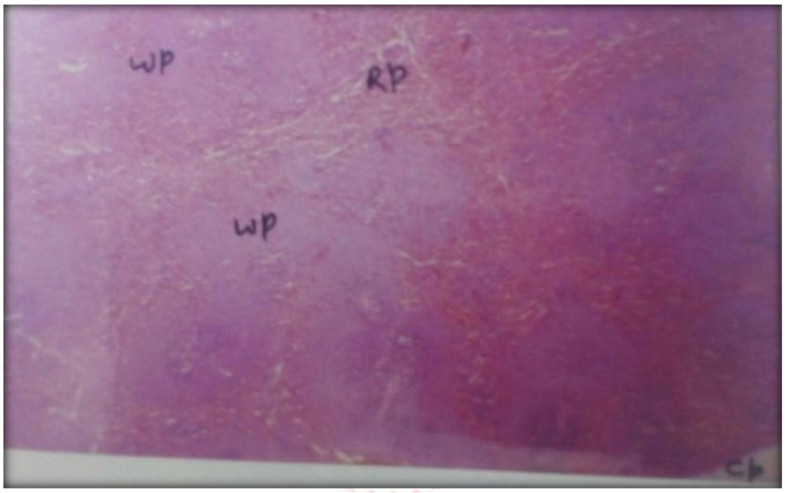

Fig. 2b: Photomicrograph of section of spleen from lower dose *Karanja* Seed administered SRBC sensitized group

(I X 32 magnification) Wp: white pulp Rp: red pulp Cp: capsule (Note : increased proportion of white pulp)

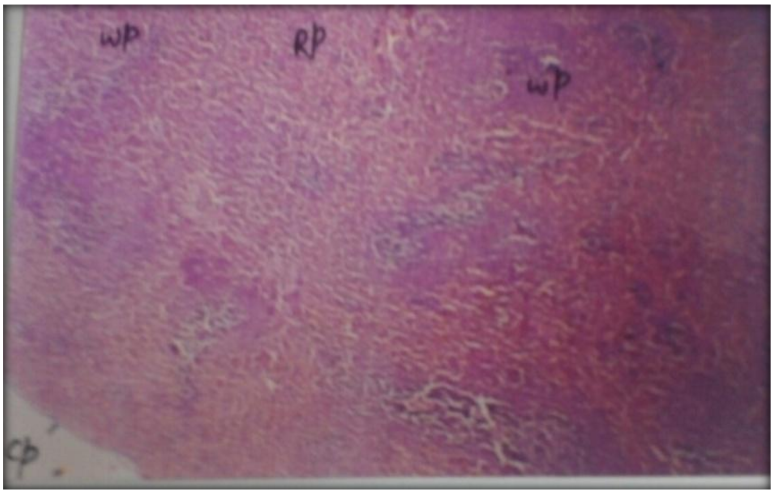

Fig. 2c: Photomicrograph of section of spleen from higher dose *Karanja* Seed administered SRBC sensitized group.

(I X 32 magnification) Wp: white pulp Rp: red pulp Cp: capsule (Note : Normal cyto-architecture)

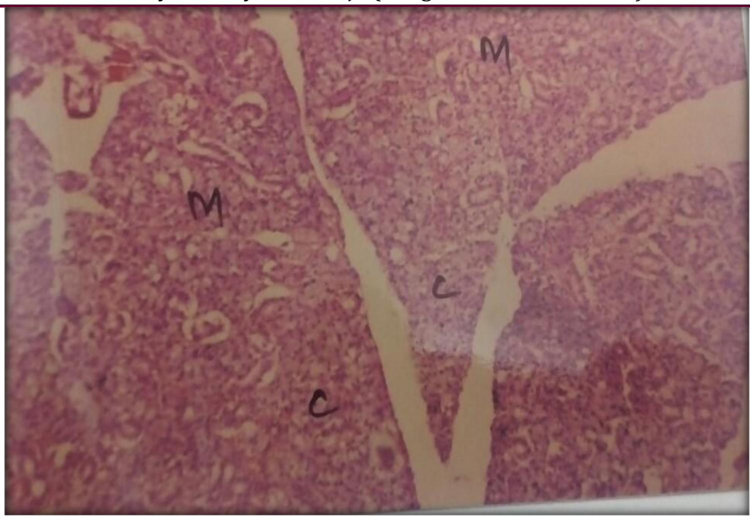

Fig. 3a: Photomicrograph of section of thymus from control SRBC sensitized group.
(I X 100 magnification)
C: Cortex M: Medulla; B.V.: Blood vessel (Note : Normal cyto-architecture)

Fig. 3b: Photomicrograph of section of thymus from lower dose *Karanja* Seed administered SRBC sensitized group. (I X 32 magnification) control SRBC sensitized group.
C: Cortex M: Medulla; B.V.: Blood vessel

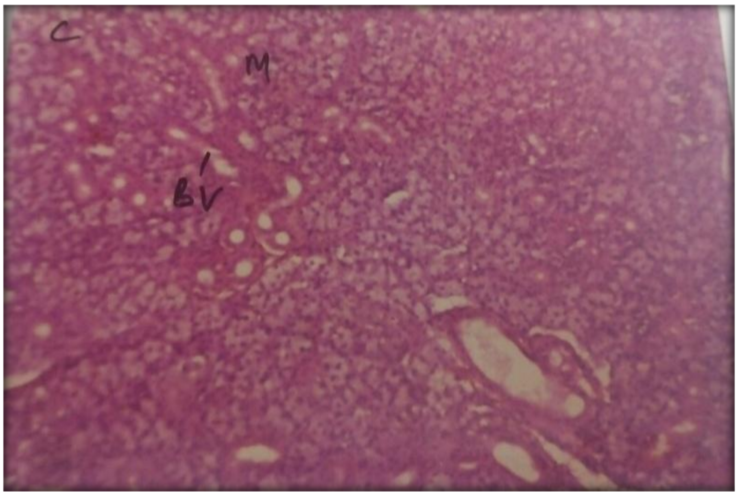

Fig. 3c: Photomicrograph of section of thymus from higher dose *Karanja* Seed administered SRBC sensitized group. (I X 100 magnification) control SRBC sensitized group.
C: Cortex M: Medulla B.V.: Blood vessel (Note : increased cellularity)

REFERENCES

1. Essentials of medical pharmacology KD Tripathi 6th Edition published by Jaypee brothers medical publishers (p) limited, 2008; p.no. 3.
2. Indian Medicinal Plants forgotten healers, a guide to Ayurvedic Herbal Medicine by Dr.Prakash Paranjpe, published by Chaukhamba Sanskrit Pratishthan, reprint edition, 2005; 2012: 124-125.
3. Immunomodulation –last edited on 5 August 2016, at 22:13, available from-Wikipedia. https://en. wikipedia.org/wiki/ Immuno-modulation.
4. experimental studies - Shodhganga *shodhganga.* inflibnet.ac.in/bitstream/10603/2314/12/12_part % 202.pdf
5. Methods of biostatistics for medical students and research work by B K Mahajan revised by Arun Bhadra Khanal Chapter 9 Rajkamal Electric press Reprint 2007 p.no-134.
6. histologylab.ccnmtl.columbia.edu/Histology Lab Manual.pdf
7. M Fereidoni, A Ahmadiani, S Semnanian, An accurate and simple method for measurement of paw edema, journal of pharmacological and toxicological methods, Elsevier, page: 11-14.

Cite this article as:
Gupta Chandni, Upadhayaya Ashwani. To Study Immunomodulatory Efficacy of Karanja (Pongamia Pinnata Pierre) Seed Churna in Swiss Albino Mice. AYUSHDHARA, 2016;4(3):1144-1150.

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: AYUSHDHARA is solely owned by Mahadev Publications - A non-profit publications, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. AYUSHDHARA cannot accept any responsibility or liability for the articles content which are published. The views expressed in articles by our contributing authors are not necessarily those of AYUSHDHARA editor or editorial board members.