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Studies on immunomodulatory effects of *Salacia chinensis* L. on albino rats

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ABSTRACT

India has a very rich diverse faunal and floral wealth spread across the length and breadth of country. Biodiversity hotspots like Himalayan region and Western Ghats are bestowed with innumerous number of potential medicinally important plants whose scientific research are yet to be taken up. *Salacia chinensis* is one among them and forms one of the known ethanobotanically used herbal drug for diabetes and aliments. A scientific study on physiological effect of *Salacia chinensis* can give baseline information about potency of plant drug formulae. Therefore this plant was taken up for the study of immunomodulatory effects on swiss albino rats. Rats were fed with a concentration of 1 mg/kg, 2 mg/kg, 4 mg/kg and 32 mg/kg body weight of aqoues extract of *Salacia chinensis* for a period of 14 days. Various hematological, serological and immunological parameters were studied at the end of 14days trial and compared with control group. Total leukocyte count, neutrophil percentage, blood glucose hemoglobin, hemagglutination antiboby titer against SRBC and delayed hypersensitivity reaction were found increased along the animals treated with 1mg/kg body weight of animal. On the contrary higher concentration of drug have reduced immune response compared to control group showing the negative effect of the higher concentration of drug on the immune response. The present study of *Salacia chinensis* evidently shows that concentration of 1mg/kg can boost the immune system and at the same time if used at higher concentration can determine the immune system.

INTRODUCTION

The use of plants for medicinal purposes has a very long and unbroken history in the Indian subcontinent. The "Aushadhisuktha" in the Rhigveda is the oldest document available on medicinal plants. It briefly describes the morphological character of medicinal plants, their habitat, their therapeutical classification and their uses in various ailments. In the early stages the science of medicine developed around those plants which had curing properties. The continued search for medicinal plants during the last several centuries has given rise to a long list of medicinal plants which are of great use in the treatment of diseases and for promoting health.

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The Western Ghats (8^o 20' - 20^o 40' N and 73^o - 77^o E) extending from Tapti in Gujarat to Kanyakumari in Tamil Nadu, traversing through Maharashtra, Goa, Karnataka and Kerala along the west coast and forming a practically unbroken relief for about 1600 km, with the exception of the Palghat Gap, are a magnificent stretch of hill ranges presenting rich and varied flora and fauna. Different types of vegetation occur here namely, scrub jungle, grasslands at lower altitudes, moist and dry deciduous forests, tropical evergreen forests and montane grasslands and sholas. About 5000 species of the estimated 17000 species of the flowering plants of India are found in the Western Ghats and almost all have one or the other medicinal properties (Nayar, 1996). It is also one of the 25 'Hotspots of Biodiversity' identified in the world (Myers *et al*., 2000).

A large proportion of the plants found here *viz*., 54 genera and 1720 species and 135 infraspecific taxa are endemic (Shetty and Kaveriappa, 1991). Nearly a third of the endemic taxa found here are rare or threatened and several are believed to be extinct or at serious risks of becoming extinct. The Indian subcontinent is enriched by a variety of flora. This is due to the wide diversity of climatic conditions in India ranging from desert to swamp lands. Numerous types of plants have been well recognized and categorized by botanist from the high ranges of the Himalayan tract up to sea shore of Kanyakumari. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional system of medicine.

The Western Ghat is famous for its herbal wealth. Many folklore practitioners of Dakshina Kannada district are using herbal wealth of Western Ghats for the human health and also cure their diseases. There are many plants such as *Saraca asoca* (Roxb.) Wilde, *Salacia* spp., *Pajanelia* sp., *Bridelia scandens* (Roxb.) Willd., *Breynia vitis-idaea* (Burm. f.) C. Fischer, *Holigarna arnottiana* Hook. f., *Adenanthera pavonina* L. are used by the folk healers of Dakshina Kannada district for various diseases such as skin disease, diabetes, asthma, arthritis etc., among these plants some plants are having antioxidant and immunomodulatory properties.

Traditional folklore medicines play an important role in health services around the globe. Ayurveda, the traditional system in India describes certain plants, which strengthen the host immune system. The modulation of immune response to all elevate diseases has been of interest for many years. The concept of immunomodulation relates a non specific activation of the immune system. It primarily implies a non antigen dependent stimulation of function and efficiency of macrophages, granulocytes, complement. NK cells, lymphocytes and also the production of various effector molecules by activated cells. An immune-modulator is a substance, biological or synthetic which can stimulate, suppress or modulate any of the components of the immune system. There are two types of effects-immunostimulation and immunosuppression. Most drugs however, do not have effects on only one receptor, so an immunomodulator may be at the same time an immunosuppressant and an immunostimulant on different targets within the immune system. Products that are not single chemical entities, such as herbal extracts and impure products, may have even greater plurality of effect. Many species of plants, depending on the specific extraction conditions used, have immunomodulatory effects.

Lymphocytes are one of many types of white blood cells produced in the bone marrow by the process of hematopoiesis. Lymphocytes leave the bone marrow, circulate in the blood and lymphatic system, and reside in various lymphoid organs. Because they produce and display antigen binding cell surface receptors, lymphocytes have the defining immunologic attributes of specificity, diversity, memory, and self/nonself recognition. The two major populations of lymphocytes are B lymphocytes and T lymphocytes (Goldsby *et al*., 2000). Phansalkar *et al.* (1996) studied immunomodulatory effect of *Withania somnifera* (L. Dunal) in mice with myclosuppression induced by one or more of the three compounds like cyclophosphamide, azathioprin or prednisolone by hematological and serological test. Li *et al*. (1997) studied the immunomodulatory effect of *Achyranthes bidentata* polysaccharides and showed that the polysaccharide may prime and trigger M phi and has restorative effects on the deficiency of the immune system associated with aging in mice and rats. Various investigators studied the immunomodulatory effect of different solvent extracts of plant species in mice (Matsuda *et al*., 1998; Amirghofran *et al.,* 2000; Latha *et al.,* 2000; Fulzele *et al*., 2003; Cariddi *et al.,* 2005*; Gayathri et al.,* 2005*; Gambhe et al.,* 2006*; Abhishek et al*., 2008*;* Ismail *et al.* (2009)*;* Bafna *et al.,* 2010*;* Shendige *et al.,* 2010; Vinay *et al.,* 2010).

Salacia chinensis L. is a straggling shrub with deep yellow colored root occurring throughout India including Andaman Islands. They are common in sacred groves and along hedges. Roots of the plant are acrid, bitter, thermogenic, urinary astringent, and anti-inflammatory. Traditionally they are useful in diabetics, inflammations, leprosy, skin diseases and wounds. The root bark contains proanthocyanidins consisting of monomeric leucopetargonidin, its monomer, dimer, tetramer and triterpenoids. In case of *Salacia chinensis* L. the literature on any of its medicinal and biochemical properties are not available when searched in database. However, one literature pertaining to its genotoxicity was documented. Govindarag *et al*. (2009) reported on their genotoxicity studies of mangiferin from *Salacia chinenesis L.* on *Salmonella typhimurium* TA97a, TA98, TA100, TA102, TA1535 strains with or without metabolic activation and concluded that no mutagenecity was recorded up to 5 mg/plate.

Each and every plant in wild possesses one or more medicinal properties irrespective of its parts. Most of the plants are generally used without isolating the active principle in the traditional system. Some important drugs were isolated after knowing the active principle and its properties. Presently many herbal drugs are being employed in treatments of diseases such as diabetes, cancer, asthma and skin diseases. One among them is *Salacia chinensis* which is used as an antidiabetic formulation.

With this ethnobotanical information available, a preliminary work was undertaken to study the possible immunomodulatory properties of *Salacia chinensis*. With above background, the study was taken up with the following objectives.

1. To estimate the haematological and serological parameters of the albino rats upon administration of *Salacia chinenesis* water extract.
2. To study the cell mediated immune response of albino rats for *Salacia chinensis* drug administration
3. To study the humoral immune response of albino rats for *Salacia chinensis* drug administration

MATERIALS AND METHODS

Collection of samples

Botanical distribution

Salacia chinensis belonging to the family Celastraceae. It is a straggling shrub with deep yellow coloured root, leaves up to 7.5 X 3 cm, oblong or ovate, cerenate – serrate, obtusely - acuminate at apex, coriaceous, glabrous, flowers 6 mm across, honey – scented, fascicled on axillary tubercles, pedicels *ca* 12 mm long. Calyx has puberulous outside. Petals are yellowish. Stamens reflexed when the flower is open over the conical disk. Berry is globose, *ca* 1cm across, 1-seeded and red when ripe (Plate 1). Commonly known as Chinensis salacia (Eng.), Saptarangi (Hin.), Ekanayakam (Kan.), Sapthachakra (San.). Found distributed throughout India including Andaman Islands. The plant is common in sacred groves and along hedges.

Properties and Chemical constituents

Fruits are edible, roots are acrid, bitter, thermogenic, urinary astringent, anti-inflammatory and stomachic. They are useful in diabetics, inflammations, leprosy, skin diseases and wounds. The root bark contains proanthocyanidins consisting of monomeric leucopetargonidin, its monomer, dimer, tetramer and triterpenoids.

The root of *S. chinensis* (Plate 2) were collected from Shobhavana, a botanical garden present in Mijar, Moodbidri, attached to Alva's Education Foundation, Moodbidri. The roots of the same shade were dried, powered and stored in air tight container at room temperature.

Preparation of water extract Salacia chinensis

About 80 g of powdered sample was mixed with 800 ml of distilled water and boiled for 45minutes. It was then filtered and the filtrate was kept in water bath for evaporation. The concentrated extract was then transferred to pre-weighed china dish. The extract was allowed to dry. The dried extract was weighed and stored in a deep freezer maintained at -10^{o}C.

Animals

Random bred Swiss albino rats (100-150 g body weight) of both sexes were used for immunological studies. Animals were randomly divided into various treatment groups (minimum 6 animals per group) based on the concentration of the drug administered. Animals were maintained in cages with paddy husk as bedding. Animals were housed at temperature 24 ± 2^{o}C, 12 hour light or dark cycle and fed with standard pellet diet and water ad libitum (Plate 3).

Dosage

The animals were divided into five groups. Each group comprised of a minimum of 6 animals. The plant extract was suspended in water and was administered orally for 14 days. Group I (control) received water; group II plant extract @ 1 mg/kg body weight; group III, @ 2 mg/kg; group IV, @ 4 mg/kg and group V received plant extract @ 32 mg/kg body weight of the animal. For animal experiment methods of Bin-Hafeez *et al*. (2003) was followed. The dose volume was calculated to be not more than 1 ml of drug preparation per animal. Control animals received 1 ml of water.

Immunization

Sheep RBC (SRBC) were collected in Alsever's solution, washed three times in phosphate buffered saline (PBS) and adjusted to a concentration of $0.5X10^9$ cells/ml. On the 7th day all the rats were immunized with $0.5X10^9$ cells/ml of SRBC peritonially using an insulin syringe. This is considered as the first dose. On the 11th day a booster dose was given.

Alsever's solution

Dextrose	-	2 g
Trisodium citrate dehydrate	-	0.8 g
Citric acid monohydrate	-	0.055 g
Sodium chloride	-	2.1 g
Distilled water	-	100 ml

Phosphate buffered saline

Sodium chloride	-	8 g
Potassium chloride	-	0.2 g
Disodium hydrogen phosphate	-	1.15 g
Potassium dihydrogen phosphate	-	0.2 g
Magnesium chloride	-	0.1 g
Calcium chloride	-	0.1 g
Distilled water	-	100 ml

Body weight and lymphoid organ weight

The animals were humanized after 14th day of treatment. Before sacrificing the animals, body weight of all the animals were recorded and blood was collected by heart puncturing using 21 gauge needle and syringe. The collected blood was used for performing different tests such as total haemocyte count, differential count, blood glucose and hemoglobin. The serum was also prepared to run an assay for hemagglutinating antibody titre and serum albumin and globulin.

After collecting blood, animals were sacrificed and the weight of organs like liver, thymus and spleen were recorded. Lymphoid tissues like, spleen, thymus including liver and kidney were fixed in phosphate buffered formalin for histological investigations to be conducted as a continued part of this work in a later period.

Phosphate buffered formalin

40% Formaldehyde	-	100 ml
Distilled water	-	900 ml
Sodium dihydrogen phosphate	-	4 g
Disodium hydrogen phosphate	-	6 g

Blood glucose test (Trinder's method)

Three test tubes were taken and marked as B (blank), T (test), and S (standard). 1 ml of glucose solution was added to all

the tubes. To the tube 'T' 0.01 ml of sample plasma was added. 0.01 ml of standard glucose was added to the tube S. All the tubes were incubated at 37^{o}C for 5 minutes. Absorbance was read at 630 nm in a UV spectrophotometer (Elico, SL159).

Hemoglobin test (Cyanmethemoglobin method-CMG method)

As much as 5 ml of Drabkin's reagent was added to the tubes marked as B (blank), S (Standard) and T (Test). Exactly 0.02 ml of blood was added to the tube T. CMG standard up to a volume of 0.02 ml was added to the tube S. Then 0.02 ml of distilled water was added to the tube B. All the tubes were incubated at room temperature for 10 minutes. The absorbance was read at 540 nm in UV spectrophotometer.

Determination of total serum protein and albumin : globulin ratio

This was estimated by Biuret method and absorbance was read at 540 nm in a UV spectrophotometer.

Total leukocyte count

It was determined by WBC diluting fluid using Hemocytometer.

Differential count of white blood cells

Blood smear was prepared on a clean glass slide. Smear was allowed to dry and was fixed in methanol for 3 minutes. Slide was dried and dipped in Field's solution B for 5 seconds. It was then rinsed with water and allowed to dry. Then it was stained with Field's solution A for 15 seconds. After staining slide was rinsed with water and dried again. Stained slide was then observed under microscope.

Hemagglutination antibody titer (Bin-Hafeez *et al.*, 2001)

As much as 100 µl of serum was heat inactivated at 56^{o}C in water bath for 30 minutes. About 50 µl of PBS was added to all 12 wells of microtiter plate row. First well was taken as control and was not added with serum, instead it received only PBS. Next well received 50µl of heat inactivated serum. From the same well using a micropipetter, 50µl of the mixture was taken after completely mixing it with the pipette and is serially diluted by 2 fold in the subsequent wells. Finally 50 µl of SRBC with a cell density of $0.5x10^{9}$/ml was added to all the wells. The plate was gently tapped to mix the cells and was incubated at 37 ^{o}C for 2 hours. The value of antibody titer was assigned to the highest serum dilution showing at least 50% of visible hemagglutination.

Hypersensitivity reaction

On the day of termination of the treatment, animals were sensitized with $0.025x10^{9}$ cells/ml of SRBC on the right hind footpad by gently injecting the same using a insulin syringe. Increase in footpad thickness of rat was measured after 3 hours of the treatment and increase in volume of foot pad was measured manually.

RESULT

During the study various morphometric, haematological, serological and other parameters required to assess the immune status of the albino rats upon administration of *Salacia chinensis* drug formulation for 14 days were recorded. The body weight of the animals of control group and that of treatment groups are given in the table 1. The table also shows the weight of different vital lymphoid organs taken at the time of humanization after 14 days of treatment. The body weight of the animals showed constant in the case of lower concentration of the drug (1 mg/kg). However, at higher concentration, the animals showed reduced body weight, with more reduction in highest concentration. Similarly for the vital organs like liver, the higher dose showed increased mass of liver when compared to lower doses of the drug.

Table. 1: Morphometric measures of albino rats treated with extracts of *Salacia chinensis.*

Groups	Body weight (g)		Weight of the organs (g)		
	Initial weight	Final weight	Liver	Thymus	Spleen
Control	100	100	2.58	0.02	0.42
Group I (1 mg/kg)	100	100	2.68	0.02	0.26
Group II (2 mg/kg)	100	75	1.93	0.02	0.42
Group III (4 mg/kg)	100	70	1.98	0.02	0.17
Group IV (32 mg/kg)	110	70	3.23	0.02	0.45

The total leukocyte count and differential count are showed in table 2, fig. 1 and 2. When compared to control group total leukocyte count was found increased in the lowest concentration (1 mg/kg) of drug administration. However increased in the drug concentration has further decreased the total leukocyte count. The percentage of neutrophils were found highest in lowest concentration (1 mg/kg) of drug tested with lowest percentage in highest concentration of a drug.

Table. 2: Hematological parameters of albino rats treated with extracts of *Salacia chinensis* .

Groups	Total leukocyte count (%) (WBC/mm^3)	Total differential count (%)				
		L	M	N	E	B
Control	2050	40	4	52	3	1
Group I (1 mg/kg)	2750	33	6	57	3	1
Group II (2 mg/kg)	2600	44	6	47	2	1
Group III (4 mg/kg)	2450	40	6	50	3	1
Group IV (32 mg/kg)	2300	37	5	44	3	1

Similar to other parameters glucose level is also found to be at higher concentration of 123 mg/dl in lowest concentration group (1 mg/kg). Whereas increased concentration of the drug had reduced the blood glucose level. Similar trend is noticed for hemoglobin, serum protein(Plate 4) and albumin globulin ratio.

Hemoglobin is found to be highest in group I where drug administration was 1mg/kg (table 3 and fig. 3).

Table. 3: Serological parameters for albino rats treated with varying concentrations of extracts of *Salacia chinensis* .

Groups	Percentage of glucose(mg/dl)	Percentage of Hemoglobin (g/dl)	Serum protein (g/100ml)	Albumin globulin ratio
Control	93	5	7	6:1
Group I (1 mg/kg)	123	8.9	8.5	8:1
Group II (2 mg/kg)	107	6.8	7.5	7:2
Group III (4 mg/kg)	102	4.6	7.2	7:1
Group IV (32mg/kg)	79	4.2	7.2	7:1

The results of HA titer is given in the table 4 figure 4. After the immunization of the animals both for the control and treatment groups hemagglutination antibody titer were estimated. Among the animals of group I and II which received drug at concentration 1 mg/kg and 2 mg/kg respectively showed highest antibody titer against SRBC. However the increased concentration of drug administered did not show proportionate increase in the hemagglutination antibody titer (Plate 5). Delayed hypersensitivity reaction was noted highest in group I which received the lowest concentration (1 mg/kg) of drug when compared to control group. In this study a remarkable increase in paw volume was seen in case of drug administered at the rate of 350 mg/kg/day (Plate 6). In case of other groups hypersensitivity reaction could not be distinguished from that of the control group (Table 4 and fig. 5).

Table. 4: HA titer and delayed type hypersensitivity response of albino rats treated with extracts of *Salacia chinensis.*

Groups	Hemagglutination antibody titer	Hypersensitivity reaction (% increase in paw volume)
Control	1:4096	10
Group I (1 mg/kg)	1:65536	17
Group II (2 mg/kg)	1:65536	10
Group III (4 mg/kg)	1:1024	10
Group IV (32 mg/kg)	1:256	10

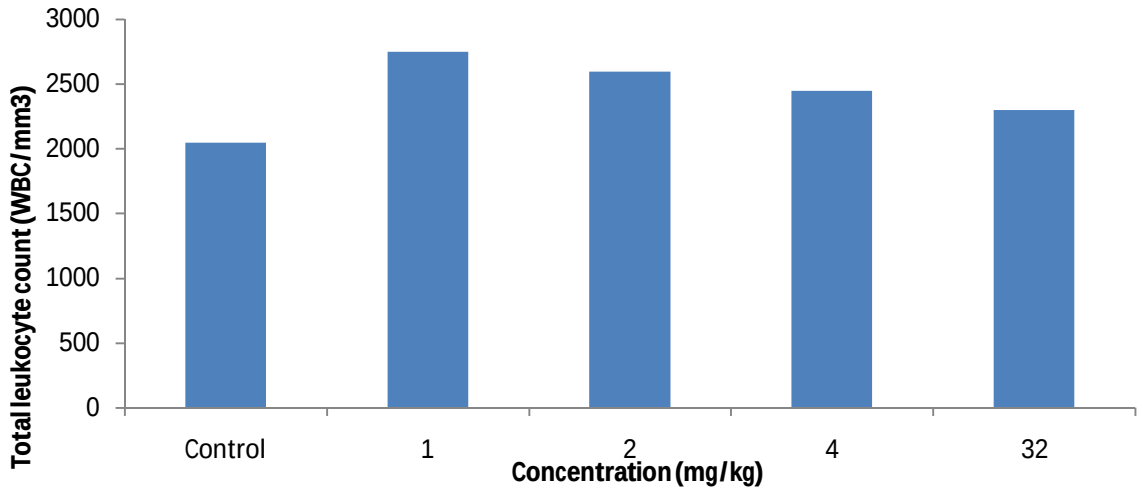

Fig. 1: Total leukocyte count of albino rats fed with extracts of *Salacia chinensis.*

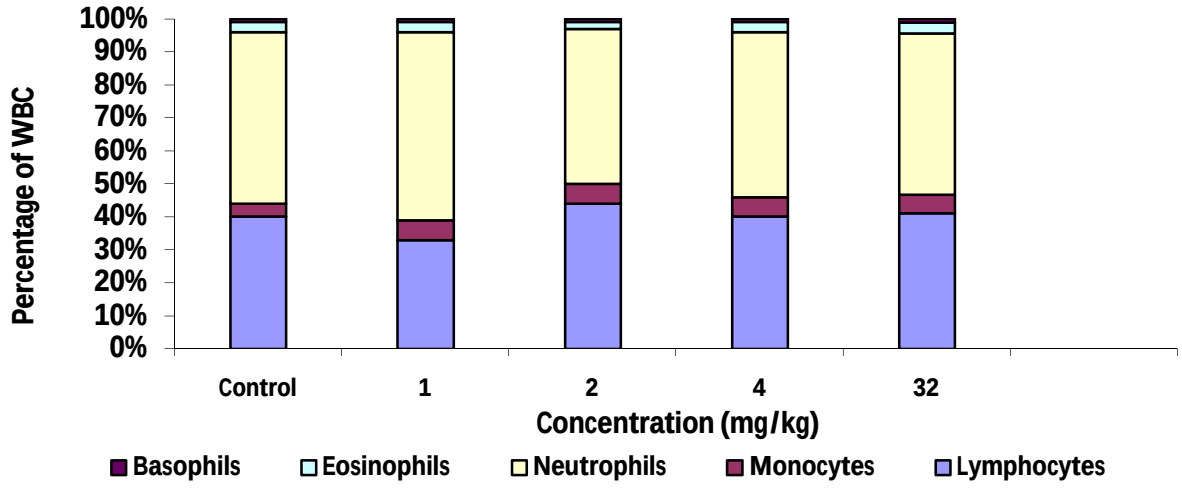

Fig. 2: Differential count of albino rats fed with extracts of *Salacia chinensis*

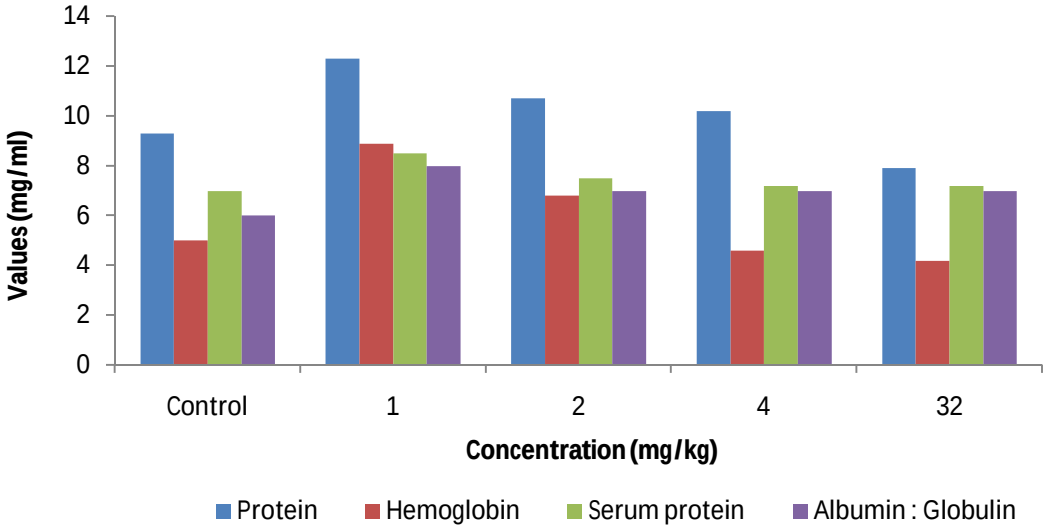

Fig. 3: Serological parameters for albino rats fed with varying concentration of extracts of *Salacia chinensis* L.

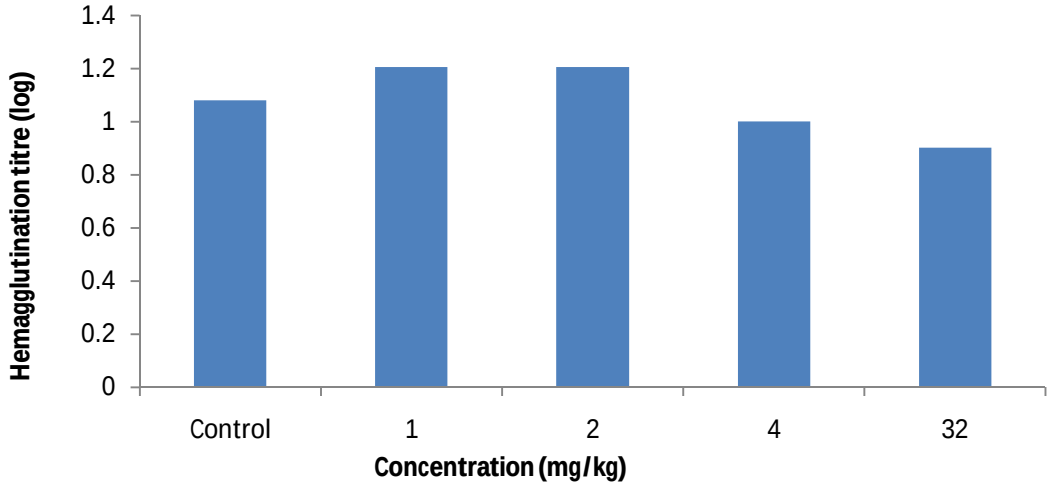

Fig. 4: HA titer of albino rats fed with extracts of *Salacia chinensis* L.

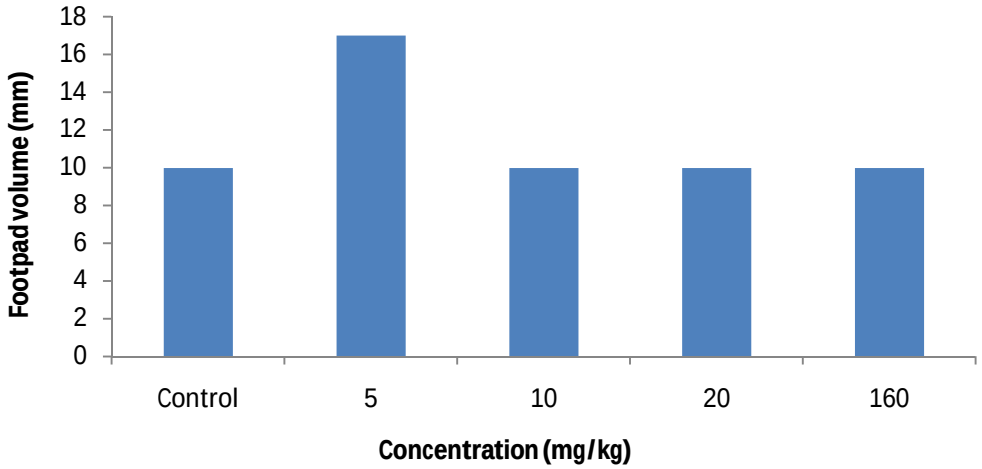

Fig. 5: Hypersensitivity of albino rats fed with extracts of *Salacia chinensis* L.

Plate. 1: *Salacia chinensis* plant.

Plate. 2: *Salacia chinensis* root.

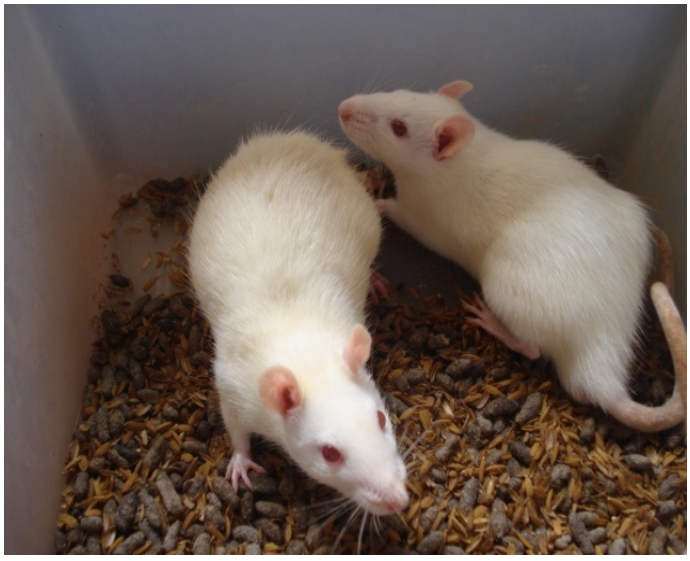

Plate. 3: Swiss albino rats.

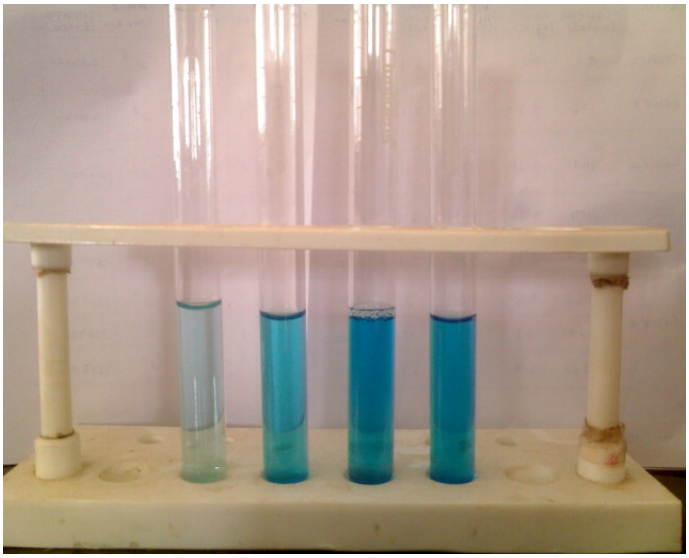

Plate. 4: Tubes showing result of serum protein .

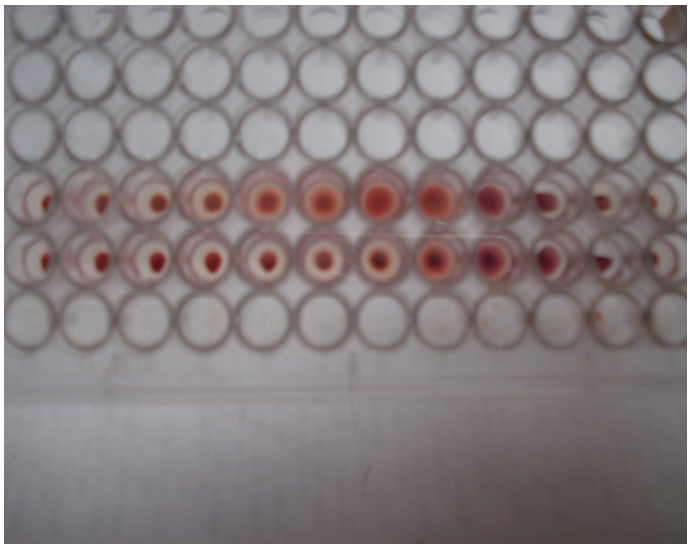

Plate. 5: Titer plate showing Hemagglutination reaction.

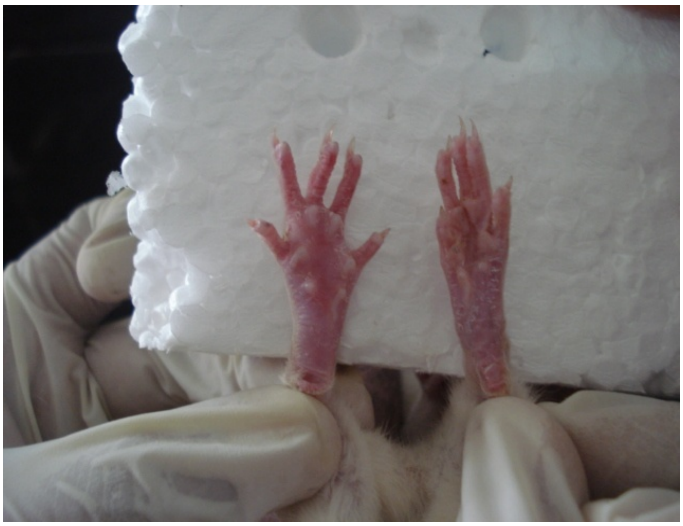

Plate. 6: Foot pad showing increase in paw volume (Hypersensitivity response).

DISCUSSION

Traditional folklore medicine derived from herbs have been used for centuries in Indian subcontinent which harbors a wide range of medicinally important plants distributed throughout Western Ghats and Himalayas. Though thousands of plants have the potential to be used as medicine only few plants have been explored scientifically to elucidate its medicinal properties. However, ethnobotanical knowledge of Indians on medicinal use of those plants is vast and is ever increasing. Though traditionally their medicinal potency is proven they need to be scientifically strengthen with research data conducted on such plants in a laboratory set up. *Salacia chinensis* L. is one of such plant where people have been using it for centuries as antidiabetic drug. Literature on this plant on any other health benefit effect or immunomodulatory activities is not available. Therefore the present findings on the Immunomodulatory studies deduced that, the albino rats administered with varying concentrations of aqueous extracts of *Salacia chinensis* would give a base line data and scientific evidence of immunological properties of the chosen plant.

Salacia chinensis aquoes extract when administered to the albino rats at varying concentration have shown effect on their body weight and relative weight of vital organs like liver. The body weight remain constant (during 14 days trial) among the group 1 which received 1 mg/kg of test drug. However with increase in concentration of the drug there was gradual reduction in the body weight indicating that drug is growth retarding instead of growth enhancing at concentration above 2 mg/kg body weight. The results are also strengthened with data obtained from liver which is a vital organ that respond immediately to any drug or toxin. In the present study the highest concentration (32 mg/kg) showed the highest liver mass showing possible hyperactivity at liver to ameliorate the toxic effect of the drug administered. Incidentally the lower concentration (1 mg/kg) of the drug showed the relative mass of liver to be similarly to control showing normal activity of liver. In case of thymus no difference among the control and treatment group were noted. Bin-Hafeez *et al.* (2003) studied immunomodulatory effect of fenugreek extract in mice showed that no significant in body weight gained could not be noted among the animals and author also observed no effect of spleen on the animals. Similar to our results the same author found increased mass of liver in concentration up to 100 mg/kg and however they have opined that increase in liver mass could not be correlated to any other toxic effect as revealed by assays on some enzymes. Similar result is observed by Bin-Hafeez *et al*. (2003).

One of the earliest immune response can be seen and measured by studying the hematological parameters of an animal. Accordingly parameters like total leukocyte count and differential count were measured for control group as well as group which received various concentrations of drug. Blood cells are the first cells to be respond to invading non self materials. A immunomodulatory effect of any immune substance would first seen as a change in leukocyte count and differential count. In the present study group 1 which received lower concentration (1 mg/kg) of drug showed highest leukocyte count of 2750 WBC/mm^3 showing the initial triggering of blood cell to mount a potent immune response. The results showing lowered concentration of drug (1 mg/kg) are better to elicit good immune response than higher concentrations (2mg/kg, 4mg/kg and 32 mg/kg) of drug administered. The results are further strengthened with highest percentage of neutrophil being circulated in the group receiving lower concentration (1 mg/kg) of drug.

Serum protein and serum albumin globulin ratio is one of the earliest indicators of normal serum chemistry of an individual. A change in serum protein concentration and albumin ratio would hint us about the altered immune response status of the individual. Accordingly in the present study serum protein level and albumin globulin ratio is found to be similar in case of control and higher concentration (32 mg/kg) of the drug but in the lower concentration (1 mg/kg of drug test the group showed increase in serum protein and albumin ratio showing that higher immune response might have contributed to the serum protein in terms of different molecules such as immunoglobulins and other humoral factors. Similar results were observed for glucose and hemoglobin. Hemoglobin is also one of the important parameter that would reveal the health status of the individual. So in the present case group 1 receiving the lowest concentration(1 mg/kg) of the drug show a better health index based on hemoglobin.

Hemagglutination antibody titer assay is one of the key parameter used to assess the humoral immune response of the animal. As the antigen is expected to induce the production of antiserum against it, in the present study sheep red blood cells were used to elucidate the production of antibody against RBC. In a individual where immune system is primed antibody against a particular antigen is expected to be at higher titer. Accordingly in the present study a very high hemagglutination antibody titer was recorded and group 1 and group 2 individual which received the lowest concentration (1 mg/kg and 2 mg/kg) of test drug. On the contrary higher concentrations of the drug have surprisingly reduced the HA titer. Pradhan *et al* (2009) administered extract of herbal product to albino rats showed a increased HA titer when drug was used at a concentration of 50mg/kg. Similarly Bin-Hafeez *et al* (2003) also showed increased HA tire at doses of 50 mg/kg and above, of fenugreek extract administered on mice. He recorded the HA titer up to 1:2389. Further Fulzele *et al* (2002) studying immunostimulant activity of *Ashtamangala ghrita* in rats showed that HA titer could be well employed to study humoral immunomodulatory activity among the animals treated with drug. The authors showed that up to 300mg/kg of the crude drug could enhance the humoral immune response. Upon examining the present results it is evident that the water extract of *Salacia chinensis* at concentrations less than 2 mg/kg induces humoral immune response as evidenced by HA titer.

Delayed type hypersensitivity reaction has been widely used as one of the parameter to measure cell mediated immune response of the animal. Prasad *et al* (2006) used delayed type hypersensitivity assay to evaluate immunomodulatory activity of *Momordica charantia* ghrita extract on albino rats. The authors

discovered a remarkable increase in paw volume in case of drug administered at the rate of 350 mg/kg/day. Pradhan *et. al.* (2009) also showed an increased hypersensitivity reaction in case of rats administered with herbal drugs. In the same line of earlier results, Bin-Hafeez *et. al.* (2003) also showed that when fenugreek was used at a concentration at 50 mg/kg a significant increase in delayed type hypersensitivity response was noticed when compared to control. In addition, Fulzel *et al*. (2002) showed *Ashtamangala ghrita* could also elucidate increased delayed type hypersensitivity response.

The overall results of the present study showed that the immune response is clearly boosted upon on administration of aqueous extract of *Salacia chinensis*. It is evident from the results that lower concentration of drug extract particularly 1mg/kg body weight has the potential to trigger the both humoral and cellular immune response. Contrarily 2mg/kg body weight or more has yielded a negative response in terms of mounting a immune response. Though these kinds of results have not been encountered in the literature it is not uncommon to have a varied physiological function of a plant derivative. A drug, as per the science of herbal drug can result in different action based on the concentration of the drug prescribed. It is very common in Ayurvedic practice were concentration dependent effect is achieved as per the requirement. Therefore, there is every possibility that a aqueous extract of *Salacia chinensis* might trigger a toxicological or a negative effect on any of the physiological or immunological system of the body Nevertheless the drug tested has evidently shown the immunomodulatory effect when used at lower concentration. As a concludery mark *Salacia chinensis* could be employed to boost immune system at low concentration and it should also be kept in mind that high concentration of same could lead to physiological effect in the body.

REFERENCES

Abhishek, S.S., Wakade, S.A. and Juvekar, A.R. Indian Journal of Experimental Biology 2008; 46 505- 509.

Amirghofran, Z., Azadbakht, M and Karimi, M.H. Evaluation of Immunomodulatory effects of five herbal plants. Journal of Ethanopharmacol. 2000; 72 (1) : 167-172 .

Bafna, A. and Mishra, S. Antioxidant and immunomodulatory activity of the Alkaloidal Fraction of *Cissampelos pareira* Linn. Sci Pharm 2010; 78 : 21-31.

Benencia F., Courrèges MC. and Coulombié FC. In vivo and in vitro immunomodulatory activities of *Trichilia glabra* aqueous leaf extracts. J Pharmacol. 2000; 69(3):199-205.

Bertagnolli, M.M. Chapter 1: Introduction: functions of the immune system. Cytokines and T lymphocytes, therapeutic manipulation of the immune system. Armstrong Printing, Texas (1993) pp. 4-7.

Bin-Hafeez, B., Haque, R., Parvez, S., Pandey, S., Sayeed, I and Raisuddin, S. Immunomodulatory effect of *Trigonella foenumgraecum* L. extract in mice. Internatl. Immunopharmacol. 2003; 3 : 257-265.

Bin-Hafeez, B,Ahmad, I, Haque, R. and Raisuddin, S. Protective effect of *Cassia occidentalis* L. on cyclophosphamide -induced suppression of humoral immunity in mice.J Ethnopharmacol. 2001;75:13-18.

Bonacorsi, C., Raddi, M.S.G., Carlos, I.Z., Sannomiya, M. and Vilegas, W. Anti-*Helicobacter pylori* activity and immunostimulatory effect of extracts from *Byrosonima crassa* Nied. BMC Complementary and Alternative Medicine 2009; 92.

Carrasco, Kenji, R., Martins, S. and Luiz, J. Immunomodulatory activity of *Zingiber officinale* Roscoe, *Salvia officinalis* L. and *Syzygium aromaticum* L. essential oils: evidence for humor- and cell-mediated responses. J. Pharmacy and Pharmacol. 2009; 61 (7) : 961-967.

Fulzele, S.V., Satturwar, P.M., Joshi, S.B. and Dorle, A.K. Study of the immunomodulatory activity of Haridradi ghrita in rats. Indian Journal of Pharmacol. 2002; 35 : 51-54.

Fulzele, S.V., Bhurchandi, P.M., Kanoje, V.M., Joshi, S.B. and Dorle, A.K. Immunostimulant activity of *Ashtamangal ghrita* in rats. Indian J. Pharmacol. 2002; 34 : 194-197.

Gabhe, S.Y., Tatke, P.A. and Khan, T.A. Evaluation of the immunomodulatory activity of the methanol extract of *Ficus benghalensis* roots in rats. Indian J. Pharmacol. 2006; 38 (4) : 271-275.

Gaur, K., Kori, M.L. and Nema, R.K. Comparative screening of immunomodulatory activity of hydro-alcoholic extract of *Hibiscus rosa sinensis* and ethanolic extract of *Cleome gynandra* Linn. Global Journal of Pharmacol. 2009; 3(2**)** : 85-89.

Gayathri, V., Asha, V.V and Subramoniam.A. Preliminary studies on the Immunomodulatory and antioxidant properties of *Selaginella* species. Indian J Pharmacol. 2005; 37 (6) : 381-385.

Ghaisas, MM., Shaikh, SA. And Deshpande, AD. Evaluation of the Immunomodulatory activity of ethanolic extract of the stem bark of *Bauhinia variegata* Linn. Journal of Ethanopharmacol. 2009; 3 (1) : 70-74.

Gopalakrishna, Bhat, K. Flora of Udupi. Manipal Press Limited Manipal (2003) 462.

Gupta, B.N.S.P., Pramanik, S., Tiwari, O.P., Thacker, N., Pande, M.S. and Upmanyu, N. Immunomodulatory activity of *Gymnema sylvestre* leaves. The Internatl. J. Pharmacol. 2010; 8 (2).

Govindaraj, Y., Melanaphuru, V., Agrahari, V., Gupta, S. and Nema, R.K. Genotoxicity studies of Magiferin isolated from *Salacia chinensis* Linn. Academic J. Plant Sci. 2009; 2 (3) : 199-204.

Ismail, S. and Asad, M. Immunomodulatory activity of *Acacia catechu*. Indian J Physiol Pharmacol. 2009; 53 (1) : 25-33.

Janeway, C.A, Jr., Travers, P., Walport, M., and Capra, J.D. Immunobiology: the immune system in health and disease 4th edition. New York: Garland. (1999), pp. 8, 228.

Khare, C.P. Indian Medicinal Plants. Springer Publishers. (2007), p.125.

Latha PG, Evans DA, Panikkar KR. And Jayavardhanan KK. Immunomodulatory and antitumour properties of *Psoralea corylifolia* seeds. Fitoterapia 2000; 71(3**)**:223-31.

Liang HR., Vuorela P., Vuorela H. and Hiltunen R. Isolation and immunomodulatory effect of flavonol glycosides from *Epimedium hunanense*. Planta Med. 1997; 63(4):316-9.

Li ZK. and Li DD. The immunomodulatory effect of *Achyranthes bidentata* polysaccharides. Yao Xue Xue Bao 1997; 32(12):881-7.

Matsuda, H. Shimoda, H. Yamahara J and Yoshikawa, M. Immunomodulatory activity of thunberginol A and related compounds isolated from *Hydrangeae dulcis* folium on splenocyte proliferation activated by mitogens. Bioorg Med Chem Lett 1998; 8(3) : 215-20.

Mehrotra, S., Mishra, K.P., Maurya, R., Srimal, R, C. and Singh, V.K. Immunomodulation by ethanolic extract of *Boerhaavia diffusa* roots. International Immunopharmacol. 2002; 2 : 987- 996.

Myeres, N., Mittermeir, R. A., Mittermier, C.G., da Fonseca, G.A.B. and Kent, J. Biodiversity hotspots for conservation priorities. Nature 2000; 403: 853-858.

Nayar, M.P. *Hotspots of Endemic Plants of India, Nepal and Bhutan.* Tropical Botanical Garden and Research Institute, Palode, Thiruvanathapuram (1996), pp.196

Pandima Devi, K., Ram, M.S., Sreepriya, M., Ilavazhagan, G. and Devaki, T. Immunomodulatory effects of *Premna tomentosa* extract against Cr (IV) induced toxicity in splenic lymphocytes – an *in vitro* study. Biomedicine & Pharmacol. 2003; 57 : 105-108.

Pereyra, M. L.G., Cariddi, LN., Ybarra, F., Isola, MC., Dema, MS., Sabini, L. and Maldonado, AM. Immunomodulating properties of *Minthostachys verticillata* on human lymphocytes and basophils. Revista Alergia Mexico 2005; 52 (3): 105-112.

Phansalkar, N., Ziauddin, M., Patki, P., Diwanay, S. and Patwardh, B. Studies on the immunomodulatory effects of *Withania somnifera* (L. Dunal). J. Ethnopharmacol. 1996; 50 (2) : 69-76.

Pradhan, D., Panda, P.K. and Tripathy, G. Evaluation of the immunomodulatory activity of the methanolic extract of *Couroupita guianensis* Aubl. flowers in rats. Natural Product Radiance 2009; 8 (1) : 37- 42.

Prasad, V., Jain, V. and Dorle, A.K. Evaluation of *Momordica charantia* for immunomodulatory activity. J. Plant Sci. 2006; 1(1) : 80-85.

Ramaswamy, S.N., Radhakrishna, R.M. and Govindappa, D.A. Flora of Shimoga District Karnataka. Prasaranga, University of Mysore 2001; 436-437.

Satpute, K.L., Jadhav, M. M., Karodi, R.S., Katare, Y.S. Patil, M.J., Rub, R. and Bafna, A.R. Immunomodulatory activity of fruits of *Randia dumetorum* Lamk. Journal of Pharmacognosy and Phytotherapy 2009; 1.

Shendige, E.R.B., Asad, M., Dhamangi, S.S. and Chandrakala, G.K. Immunomodulatory activity of methanolic extract of *Morus alba* Linn. leaves. Pak. J.Pharm Sci. 2010; 23 (1) : 63-68.

Shetty, B.V. and K.M. Kaveriappa. The Western Ghats - Need for Preservation. *In: Perspectives on Dakshina Kannada and Kodagu.* Mangalore University Decennial volume, Mangalagangothri, Karnataka (1991), pp. 258-272.

Spagrude, G.J, Heimfeld, S., and Weissman, I.L. Purification and characterization of mouse hematopoietic stem cells. Science 1988; 241: 58-62.

Suntararuks, S., Yoopan, N., Rangkadilok, N. and Nookabkew, S. Immunomodulatory Effects of Cadmium and *Gynostemma pentaphyllum* on Rat Splenocyte Proliferation. J Agri. Food Chem. 2008; 56 (19): 9305-9311.

Tan, B.K.H. and Vanitha, J. Immunomodulatory and Antimicrobial Effects of Some Traditional Chinese Medicinal Herbs. Current Medicinal Chemistry 2004; 11 (11): 1423-1430.

Vinay, J., Jat,R.C., Subodh, D., Sudhir, B. and Suman, J. Immunomodulatory effect of aerial part of *Tephrosia purpurea* Linn. Journal of Pharmacy Res. 2010; 3(1) : 156-158.

Yin Lu. Fan, J. Zhao, Y. Chen, S. Zheng, X. and Yin, Y. Immunomodulatory activity of aqueous extract of *Actinidia macrosperma*. Asia Pac J Clin Nutr. 2007; 16 (1): 261-265.

Zingermagel, R., and Doherty, P. Immunological surveillance against altered self component by sensitized T lymphocytic choriomeningitis. Nature. 1974; 251 : 547-548.

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