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Research Article of Evaluation of Immunomodulatory Activity of Dalbergia Latifolia on Swis Albino Mice

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Abstract:
Purpose*: To evaluate the immunomodulatory activity of ethanolic extracts of Dalbergia latifolia bark (Fabaceae) on Swiss albino mice.*
Methods:
Model *-Cyclophosphamide induced immunosuppression model and Neutrophil adhesion test. Animal used - Swiss albino mice.*
Dose –*Cyclophosphamide-30mg/kg body weight, i.p route.*
Extract-100mg/kg and 200mg/kg body weight, oral route.
The extent of protection against immunosuppression caused by Cyclophosphamide was evaluated after 14 days of drug administration, by estimating hematological parameter and neutrophil adhesion test.
Results: *Ethanolic extracts of Dalbergia latifolia bark flavonoids showedimmunomodulatory activity by increasing the depleted levels of total WBC count and RBC, % Hb, and % neutrophils adhesion.*
Conclusions: *The extract was found to be effective immunomodulatory agents.*
Keywords:*Dalbergia latifolia, Ethanolic extract, Barks, Immunomodulatory activity, Cyclophosphamide,*

I. Introduction:

The immune system is a network of cells, tissues, and organs that work together to protect the body from infection.The immune system is a remarkably sophisticated defense system that has evolved to protect animals from invading pathogenic microorganisms and eliminating the numerous types of foreign infectious agents.[1]

The overall function of the immune system is to prevent or limit infection. This is achieved either through innate or natural immunological mechanisms which essentially serve as short term first line defence or through elaborate adaptive mechanisms which are highly specific, complex, and marked by diversity and memory.When the immune system hits the wrong target, however, it can unleash a torrent of disorders, including allergic diseases, arthritis, and a form of diabetes. If the immune system is crippled, other kinds of diseases result.[2]

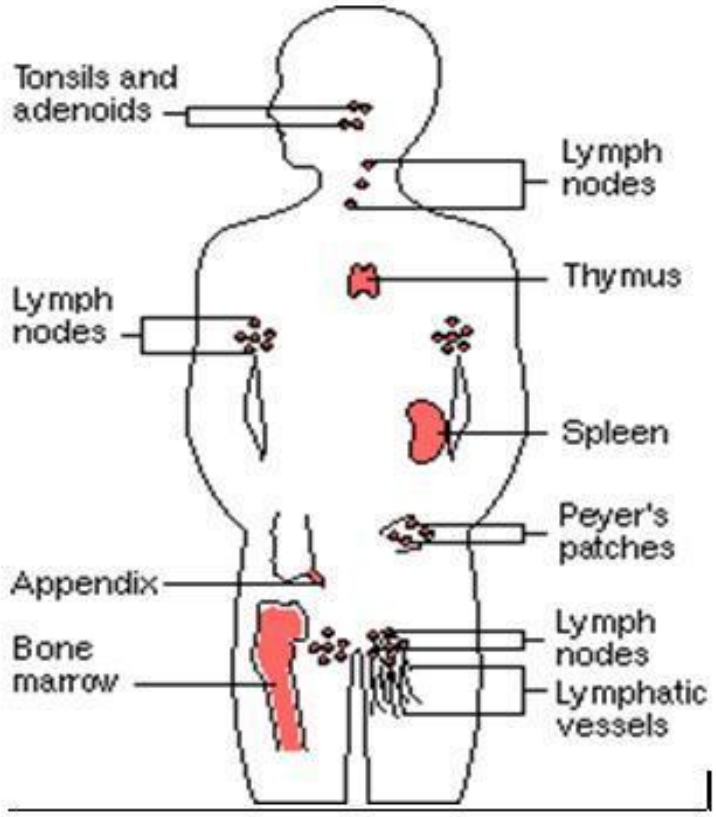
An example of this principle is found in immune-compromised people, including those with genetic immune disorders, immune-debilitating infections like HIV, and even pregnant women, who are susceptible to a range of microbes that typically do not cause infection in healthy individuals.

The Structure and function of the Immune System:

The organs of the immune system are positioned throughout the body. They are called lymphoid organs because they are home to lymphocytes, small white blood cells that are the key players in the immune system. Bone marrow, the soft tissue in the hollow center of bones, is the ultimate source of all blood cells, including lymphocytes. The thymus is a lymphoid organ that lies behind the breastbone. Lymphocytes known as T lymphocytes or T cells ("T" stands for "thymus") mature in the thymus and then migrate to other tissues. B lymphocytes, also known as B cells, become activated and mature into plasma cells, which make and release antibodies.[3]thefunction of the thymus is to produce mature T cells.The lymph nodes function as an immunologic filter for the bodily fluid known as lymph which is composed mostly of T cells, B cells, dendritic cells and macrophages, the nodes drain fluid from most of our tissues. Antigens are filtered out of the lymph in the lymph node before returning the lymph to the circulation.[4]the bone marrow produces B cells, natural killer cells, granulocytes and immature thymocyte, in addition to red blood cells and platelets.The spleen is an immunologic filter of the blood. It is made up of B cells, T cells, macrophages, dendritic cells, natural killer cells and red blood cells. In the spleen, B cells become activated and produce large amounts of antibody. Also, old red blood cells are destroyed in the spleen.[5]

The immune system is designed to protect the host from invading pathogens and to eliminate disease.**6** Activation of immune system by "non-self" antigen (alloantigen) or "self" antigen (autoantigen) is generally

believed to require processing of the antigen by the phagocytic cells such as macrophages, monocytes, or related cells.**7**



Immunomodulator are substances, which modify the activity of the immune system. They can enhance or inhibit immunological responsiveness of an organism by interfering with its regulatory mechanisms. This may be antigen independent and may directly induce production of mediators and effector molecules by the immunocompetent cells. This type of antigen independent immunity is thus distinct from one achieved by conventional immunization or by passive immunization using antibodies.**[8]**

There are more than 150 different primary immunodeficiency diseases currently recognized by the World Health Organization.[9]

Immunomodulation using medicinal plants can provide an alternative approach to conventional chemotherapy for a variety of diseases, especially when host defense mechanism has to be activated under the conditions of impaired immune response or when a selective immunosuppression is desired in situations like autoimmune disorders.**[1]**

Medicinal plants serve as therapeutic alternatives, safer choices, or in some cases, as the only effective treatment. A large number of these plants and their isolated constituents have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, and immunomodulatory effects.**[10, 11, 12, 13,14,15,16]**

Dalbergia latifolia (Roxb) Family- Fabaceae**[17]** Synonyms: Amerimnon latifolium (Roxb.) Kuntz, nom. illeg, Dalbergia emarginata Roxb.

Vernacular/common names: sitsal, beete, shisham (India); satisal (Nepal); sonokeling, sonobrits (Indonesia); palisandre de l'Inde (Fr.). Indischer Rosenholzbaum (Germ.); Indian rosewood, Bombay blackwood (Eng.); rosewood (trade name).

Dalbergia latifolia is a large glabrous tree a single stem with characteristic smell **[18]**.The tree has grey bark that peels in long fibers, compound leaves and bunches of small flowers.**[19]**

It contain dalbinol new 12a-hydroxyrotenoid **[20]**, sisafolin coumarin from seeds, - sitosterol, also contain dalbergichromene, lupeol, latifolin and dalbergin from bark of the tree, heartwood contains latinone, neoflavonoid dalcriodon**[21]** and Latinone, a substituted phenanthrene-1, 4- Quinone was isolated from Dalbergia latifolia .**[22]**

The genus consists of 300 species and about 25 species occur in India. Many species of Dalbergia are important timber trees, valued for their decorative and often fragrant wood, rich in aromatic oils**[23,24]**.Traditionally various species are reported to be used as aphrodisiac, abortifacient, expectorant,

anthelmintic, antipyretic, appetizer, allays thirst, vomiting, burning sensation, cures skin diseases, ulcers, diseases of the blood, reduces obesity, used in leucoderma, dyspepsia, dysentery, for diseases of the eye and nose, syphilis, stomach troubles, leprosy, leucoderma, scabies and ringworm [25,26].

Literature review shows that the Immunomodulatory activity of Dalbergia latifoliahas not been investigated.Hence the present study was undertaken to evaluate the effect of flavonoid of Dalbergia latifoliaon the immune system using different experimental models to substantiate the traditional claim.

Immune Mechanism

Basically there are two different types of lymphoid cells, T and B cells which mediate 'Cellular' and 'serologic' or 'humoral' immunity, respectively. Both these types of cells are Present in the circulating blood and in peripheral lymphoid tissues. The recognition of the Antigen by the T cells leads to proliferation of these cells, infiltration of immune cells at the site of action and cellular immunity. These reactions may be manifested as Delayed type Hypersensitivity, tissue graft rejection [27]. The other limb of immune system involving B cells is responsible for the genesis of specific antibodies immunoglobulins (IgA, IgD, IgE, and IgM). The Recognition of antigen (Ag) by the B cells leads to proliferation of these cells, conversion to Plasma cells and generation of specific antibodies (Ab). The specific Ab binds with the specific Antigen leading to its inactivation or even phagocytosis.[28]

Extraction of the plant material and sample preparation:

Hydro alcoholic Extraction (Distilled water: Ethanol = 2:1) of drug was carried out by hot percolation method through Soxhlet apparatus. Thereafter extract was dried using rotary evaporator and dried extract was put to the process of standardization. The percentage yield was noted as 3.70% g.

Structureoftheisolated flavonoid:

S.No	Compound		Molecular formula	Molecular weight	Melting point
	Code	Name			
1	DL-1	2,3-dihydro-2-phenylchromen-4-one	$C_{15}H_{10}O_3$	238	75-77 ^{o}C

Phytochemical analysisof successiveextract of bark ofDalbergialatifolia.

Sl. No.	**Tests**	**Results**
1	**Tests forSteroids andTriterpenes**	
	Salkowski test	+
	Libermann-Buchard test	+
	Kahlebergtest	+
2	**Tests foralkaloids**	
	Mayer'sreagent	-
	Dragandroff's reagent	-
	Hager's reagent	-
	Wagner's reagent	-
3	**Tests forSaponins (Foamtest)**	-
4	**Tests forphenolic compounds and tannins**	
	Ferric chloride test	+
	Gelatinetest	+
	Lead acetatetest	+
5	**Tests forflavonoids**	
	Sodiumhydroxide test	+
	Ferric chloridetest	+
	Shinoda's test	+
	ZINC-HCl reduction test	+
	Lead acetatetest	+

Drugs and Chemicals with sources

The fresh bark of Dalbergia latifolia was collected from Tirupati, Andhra Pradesh in the month of June 2014, identified and authenticated by Dr. K. Madhava chetty, Asst. professor, Department of Botany, Sri Venkateswara University, Tirupati. Cyclophosphamide was used as standard immunosuppressant, were purchased from MS **Ramaiah** Memorial **Hospital** Bangalore,Leishman's stain purchased from Bharath scientific, Bangalore city.

II. Methods and Animals:

Eight week-old healthy, laboratory bred, Swiss albino mice of either sex (20-25g) were purchased from central animal research NIMHANS Reg No. 12/99 Bangalore. Animals were maintained under standard laboratory conditions such as temperature 22–25^{o}C, 12 hour light/dark cycle and provided with water and pellet food ad libitum. The experiments were conducted as per the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiments on animals, India), the experimental protocol was approved by Institutional ethical committee (KCP/IAEC-2014-15).

Acute toxicity studies

Two mice were selected with a dose of 50 mg/kg orally and examined for a period of 24 h for mortality. The subsequent doses are then increased by 1.5 factors to attain maximum non-lethal and minimum lethal dose. The extract was found to be safe at the dose of 2 g/kg p.o. According to office of pollution prevention and toxics (OPPT) guidelines (http://www.epa.gov/oppts/home/guideline.htm) (Kubavat and Asdaq, 2009), 1/10th and 1/20th of the maximum safe dose (2 g/kg) corresponding to 200 mg/kg and 100 mg/kg were selected as high and low doses, respectively.

Cyclophosphamide induced immunosuppression

The animals were divided into the 4 groups containing 6 animals in each group. Group1 (Control group) received normal water for 14 days and group 2 (Challenge group) received ethanolic extract of the drug at a dose of 100mg/kg b/w for 14days, on 11th, 12th and 13th day Cyclophosphamide solution was given intraperitoneally at a dose of 30mg/kg b/w. Group 3 (Test group) received ethanolic extract of the drug at a dose of 200mg/kg body weight orally for 14 days. On day's 11th,12th and 13th day Cyclophosphamide solution was given intraperitoneally at a dose of 30mg/kg b/w one hour after the administration ofthe extract. Group 4 received only cyclophosphamide. Blood was collected, the total leukocyte count (TLC) and DLC were performed prior to and on day 3 after injection of Cyclophosphamide. The TLC,RBC and Hb (%) in treated groups were compared with the values of the control group.

Neutrophil adhesion test (Fulzele et al., 2003; Shindeet al., 1999)

The mice were pre-treated orally with vehicle or extracts for 14 days. At the end of treatment day 14, blood samples were collected from the retro-orbital plexus into heparinized vials and analyzed for differential leukocyte count (DLC).Total leukocyte counts (TLC) and differential leukocyte counts (DLC) were analyzed by fixing blood smears and staining with Field stain I and II- Leishman's stain. After initial counts, blood samples were incubated with 80mg/ ml of nylon fibers for 15 min at 37ºC. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below

Neutrophil adhesion (%) = NI u – NI t x 100/NI u

Where

NI u = Neutrophil index of untreated blood sample.

NI t = Neutrophil index of treated blood sample.

III. Results

Effectiveness of Dalbergia latifolia compound against drug induced immunosuppression

Administration of Cyclophosphamide (30 mg/kg, i.p) produced a significant decrease in the Total Leukocyte Count from 9.5±0.081 to 2.95±0.214, Control group>High dose (D+CP) >Low dose (D+CP) >CP,RBC count from 5.00±0.116 to 3.00±0.152, and % hemoglobin from 14.92±0.081 to 8.89±0.153 (P<0.01).

Where, D = Ethanolic extract

CP =Cyclophosphamide

Figure1: Showing effect of Dalbergia Latifolia compound on cyclophosphamideinduced depletion in WBC counts.
Valuesareexpressedasmean±SEM,(n=6)***p<0.001comparedwithnormalcontrolgroup. ###p<0.01 compared with cyclophosphamidegroup.

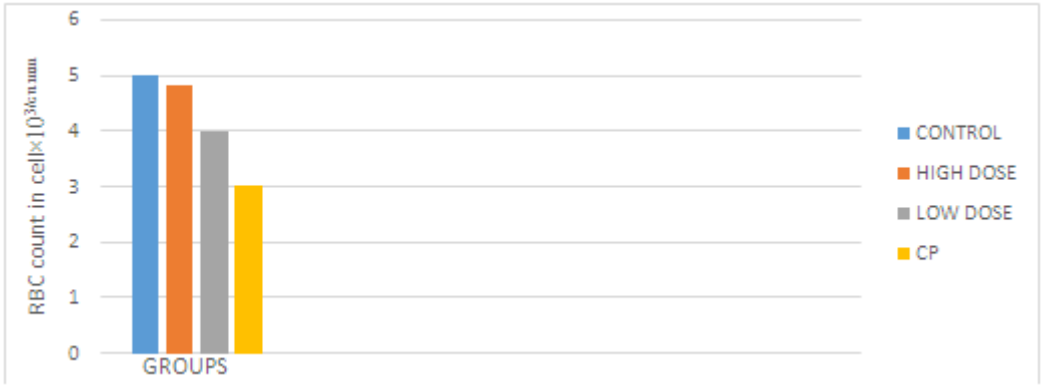


Figure2: Showing effect of Dalbergia Latifolia compound on cyclophosphamide induced depletion in RBC counts.
Valuesareexpressedasmean±SEM,(n=6)***p<0.001comparedwithnormalcontrolgroup. ###p<0.01 compared with cyclophosphamidegroup.

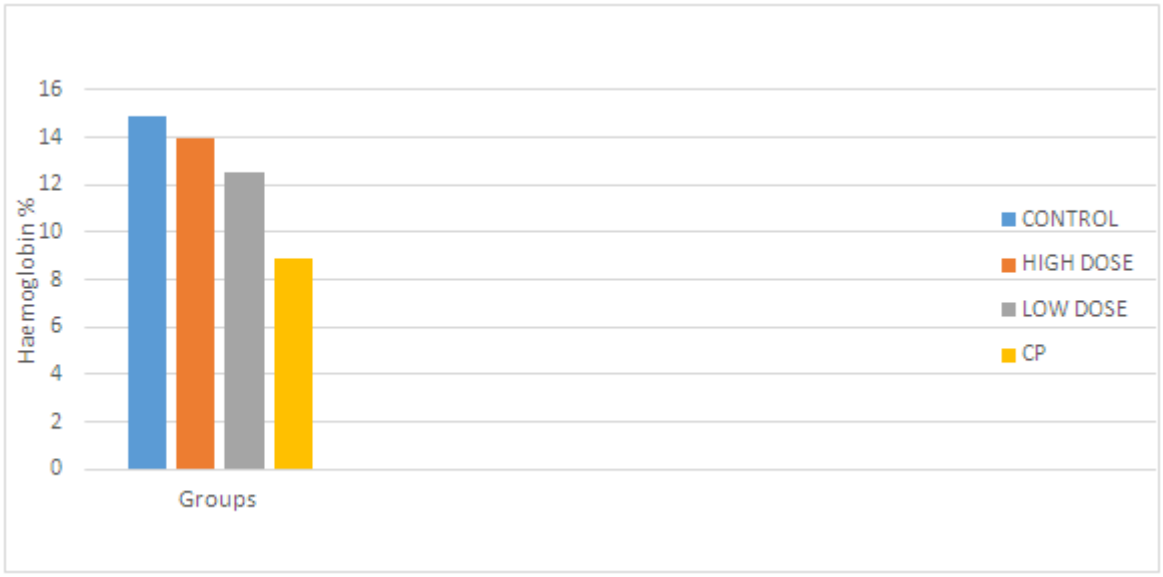


Figure3: Showing effect of Dalbergia Latifolia compound on Hemoglobin estimation

Valuesareexpressedasmean±SEM,(n=6)***p<0.001comparedwithnormalcontrolgroup. ###p<0.01 compared with cyclophosphamidegroup.

Neutrophil adhesion test

Effect of Dalbergia latifolia Neutrophil adhesion test

Treatment	TLC (10^3/mm^3) (A)		Neutrophil% (B)		Neutrophil index (A × B)		Neutrophil adhesion (%)
	UB	NFTB	UB	NFTB	UB	NFTB	
Control	8.5±0.48	8.3±0.8	30±0.1	28±0.8	255±0.96	232.4±1.6	8.86±1%
Low dose (100mg/kg)	9.6±1.2	8.8±1.2	35±1.3	30±1.3	336±2.5	264±2.5	21.42±5%
High dose (200mg/kg)	10.5±0.8	9.0±0.8	40±20	32±20	420±0.28	288±0.28	33±0.56%

All values are expressed as mean ± SEM of six observations. TLC=Total leukocyte counts
UB= untreated blood; NFTB= nylon fiber treated blood

The % neutrophil adhesion in control group animals was, 8.86±1, in low dose treated group was 21.42±5, and in high dose treated group was 33±0.56. The results of neutrophil adhesion test indicating that there was significant (P<0.001) increase in neutrophil adhesion after administration of Dalbergia latifolia ethanolic extract.

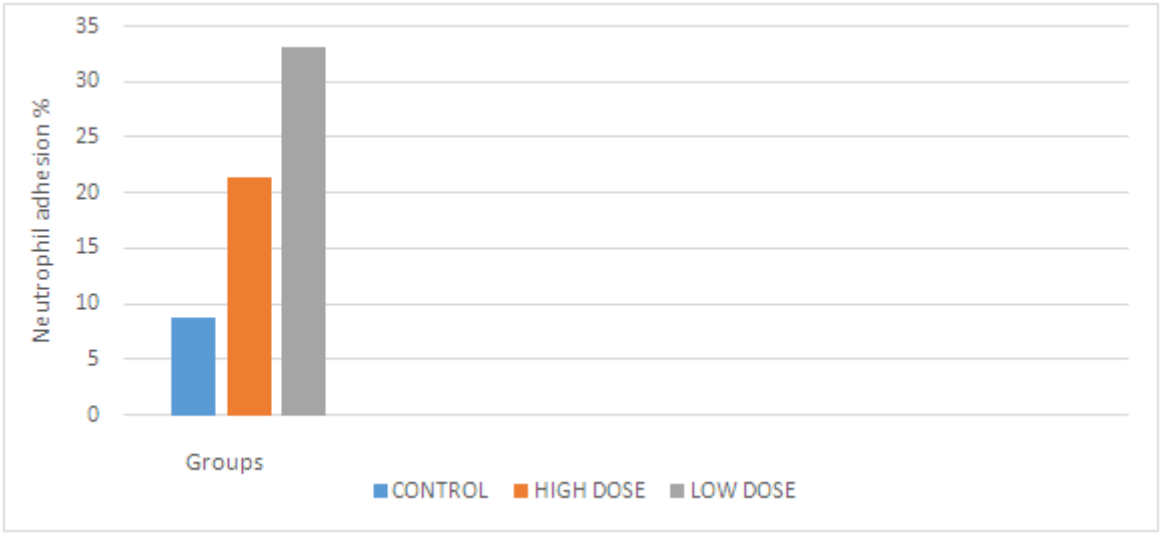


Figure3: Showing effect of Dalbergia Latifolia extracts on Neutrophil adhesion test.
Valuesareexpressedasmean±SEM,(n=6)***p<0.001comparedwithnormalcontrolgroup. ###p<0.01 compared with cyclophosphamidegroup.

IV. Discussion & Conclusion:

Immunomodulatory therapy represents an important field in the treatment of infectious diseases and is more actual every day (Nicoara and Crisan, 2003). An immunomodulator is any substance that helps to regulate the immune system.In this study we found that ethanolic extract of Dalbergia latifolia possesses immunomodulatory activity in experimental animals by increasing WBC count, RBC count, and % hemoglobin values.

In conclusion, both low dose (100 mg/kg, p.o) as well as high dose (200 mg/kg, p.o) of Dalbergia latifolia stimulates immune system by acting through cellular and humoral immunity in experimental models of immunity in animals. Increase in percent neutrophil is attributed to marginalization of phagocytic cells i.e. improved defensive response under normal circumstances.

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