

# Immunomodulatory Activity of *Dalbergia Latifolia* on Swis Albino Mice

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**Abstract:** The immune system is involved in the etiology as well as pathophysiological mechanisms of many diseases. Modulation of the immune responses to alleviate the diseases has been of interest for many years. Thus a real need exists to protect our immune systems and lead healthier lives. Hence the present study is aimed to evaluate the immunomodulatory activity of methanolic extract of *Dalbergia latifolia*. The effect of methanolic extract of *Dalbergia latifolia* on the immune system of mice was evaluated by using different experimental models such as carbon clearance test, mice lethality test. Methanolic of *Dalbergia latifolia* extract was administered orally at dose of 100mg/kg and dose of 200 mg/kg. Methanolic extract of *Dalbergia latifolia* in both doses increased the levels of serum immunoglobulin and prevented the mortality induced by bovine *Pasteur Ella multocida* in mice. On the other hand, it showed significant increase in the phagocytic index in carbon clearance assay. Hence, it was concluded that methanolic extract of *Dalbergia latifolia* increases both humoral immunity and cell mediated immunity.

**Keywords:** *Dalbergia latifolia*; Barks, Immunomodulatory activity, Levamisole.

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## I. ABOUT THE IMMUNE SYSTEM

The term immunity is derived from the Latin word “immunis”, which was originally referred to the protection from legal prosecution offered to the Roman senators during their tenures in office.

The immunity is the protection of the body from a disease caused by an infectious agent, such as bacterium or virus. The immune system is the body's defence against infectious organisms and other invaders. Through a series of steps called the immune response, the immune system attacks organisms and substances that invade body systems and cause disease. Immunity may be natural (that is, inherited) or acquired. the ability of an organism to resist disease, either through the activities of specialized blood cells or antibodies produced by them in response to natural exposure or inoculation ( active immunity) or by the injection of antiserum or the transfer of antibodies from a mother to her baby via the placenta or breast milk ( passive immunity).

The immune system is made up of a network of cells, tissues, and organs that work together to protect the body. The cells involved are white blood cells, or leukocytes, which come in two basic types that combine to seek out and destroy disease-causing organisms or substances. The cells and molecules responsible for immunity constitute the immune system, and their collective and coordinated response to foreign substances is called the immune response.<sup>1</sup>

Leukocytes are produced or stored in many locations in the body, including the thymus, spleen, and bone marrow. For this reason, they're called the lymphoid organs. There are also clumps of lymphoid tissue throughout the body, primarily as lymph nodes, that house the leukocytes.

The leukocytes circulate through the body between the organs and nodes via lymphatic vessels and blood vessels. In this way, the immune system works in a coordinated manner to monitor the body for germs or substances that might cause problems.

The two basic types of leukocytes are:

1. **phagocytes**, cells that chew up invading organisms
2. **lymphocytes**, cells that allow the body to remember and recognize previous invaders and help the body destroy them

A number of different cells are considered phagocytes. The most common type is the **neutrophil**, which primarily fights bacteria. If doctors are worried about a bacterial infection, they might order a blood test to see if a patient has an increased number of neutrophils triggered by the infection. Other types of phagocytes have their own jobs to make sure that the body responds appropriately to a specific type of invader.

The two kinds of lymphocytes are **B lymphocytes** and **T lymphocytes**. Lymphocytes start out in the bone marrow and either stay there and mature into B cells, or they leave for the thymus gland, where they mature into T cells. B lymphocytes and T lymphocytes have separate functions: B lymphocytes are like the body's military intelligence system, seeking out their targets and sending defences to lock onto them. T cells are like the soldiers, destroying the invaders that the intelligence system has identified.

#### **Here's how it works:**

When antigens (foreign substances that invade the body) are detected, several types of cells work together to recognize them and respond. These cells trigger the B lymphocytes to produce antibodies, specialized proteins that lock onto specific antigens.

Once produced, these antibodies continue to exist in a person's body, so that if the same antigen is presented to the immune system again, the antibodies are already there to do their job. So if someone gets sick with a certain disease, like chickenpox, that person typically doesn't get sick from it again.

This is also how immunizations prevent certain diseases. An immunization introduces the body to an antigen in a way that doesn't make someone sick, but does allow the body to produce antibodies that will then protect the person from future attack by the germ or substance that produces that particular disease.

#### **Autoimmune Disorders:**

Immunodeficiency disorder (PID) refers to a heterogeneous group of disorders characterized by poor or absent function in one or more components of the immune system. Over 130

Different disorders have been identified to date, with new disorders continually being recognized<sup>2,3</sup>.

In autoimmune disorders, the immune system mistakenly attacks the body's healthy organs and tissues as though they were foreign invaders. Autoimmune diseases include:<sup>4</sup>

- **Lupus**, a chronic disease marked by muscle and joint pain and inflammation (the abnormal immune response also may involve attacks on the kidneys and other organs)
- **Juvenile rheumatoid arthritis**, a disease in which the body's immune system acts as though certain body parts (such as the joints of the knee, hand, and foot) are foreign tissue and attacks them
- **Scleroderma**, a chronic autoimmune disease that can lead to inflammation and damage of the skin, joints, and internal organs
- **Ankylosing spondylitis**, a disease that involves inflammation of the spine and joints, causing stiffness and pain
- **Juvenile dermatomyositis's**, a disorder marked by inflammation and damage of the skin and muscles

#### **Allergic Disorders<sup>5</sup>:**

Allergic disorders occur when the immune system overreacts to exposure to antigens in the environment. The substances that provoke such attacks are called allergens. The immune response can cause symptoms such as swelling, watery eyes, and sneezing, and even a life-threatening reaction called anaphylaxis. Medications called antihistamines can relieve most symptoms.

#### **Allergic disorders include:**

- **Asthma**, a respiratory disorder that can cause breathing problems, frequently involves an allergic response by the lungs. If the lungs are oversensitive to certain allergens (like pollen, molds, animal dander, or dust mites), it can trigger breathing tubes in the lungs to become narrowed and swollen, leading to reduced airflow and making it hard for a person to breathe.
- **Eczema** is an itchy rash also known as atopic dermatitis. Although atopic dermatitis is not necessarily caused by an allergic reaction, it more often occurs in kids and teens who have allergies, hay fever, or asthma or who have a family history of these conditions.
- **Allergies** of several types can occur in kids and teens. Environmental allergies (to dust mites, for example), seasonal allergies (such as hay fever), drug allergies (reactions to specific medications or drugs), food allergies (such as to nuts), and allergies to toxins (bee stings, for example) are the common conditions people usually refer to as allergies.

Today's world is full of stress. Stress, basically is a reaction of mind and body against which have a significant impact on the immune response in general. The immune system is known to be involved in the etiology as well as pathologic mechanisms of many diseases. Immunology is thus probably one of the most rapidly developing areas of biomedical research and has great promises with regard to the prevention and treatment of a wide range of disorders.<sup>6</sup>

The immune system is designed to protect the host from invading pathogens and to eliminate disease<sup>8</sup>. The primary object in the past has been to suppress immune system to permit all transplantation. Activation of immune system by "non-self" antigen (alloantigen) or "self" antigen (auto antigen) is generally believed to require processing of the antigen by the phagocytic cells such as macrophages, monocytes, or related cells<sup>9</sup>.

Indian medicinal plants are a rich source of substances which are claimed to induce Para immunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions<sup>7</sup>

#### Taxonomy and nomenclature:

**Family:** Fabaceae (Papilionoideae)

**Synonyms:** *Amerimnon latifolium* (Roxb.) Kuntze, nom. illeg, *Dalbergia emarginata* Roxb.

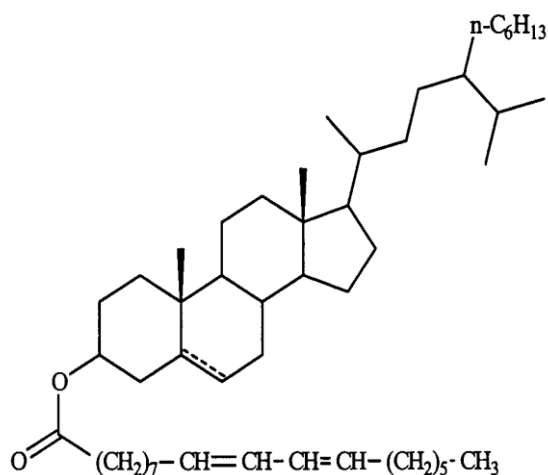
**Vernacular/common names:** sital, Beete, shisham (India); satal (Nepal); snorkelling, sonobrits (Indonesia); palisandre de l'Inde (Fr.). Indischer Rosenholzbaum (Germ.); Indian rosewood, Bombay Blackwood (Eng.); rosewood (trade name).

The genus *Dalbergia* belongs to the family Fabaceae which is one of the largest among the flowering plants, consisting of 300 species<sup>10</sup>. A large number of these species are commonly found in both the tropical and subtropical regions of the world, including Central and Southern America, Africa and Southern Asia, where they are used for the management of a wide range of disorders such as diarrhoea, dysentery, stomach ache, gonorrhoea, syphilis, scabies, leprosy and pains<sup>11,12</sup>

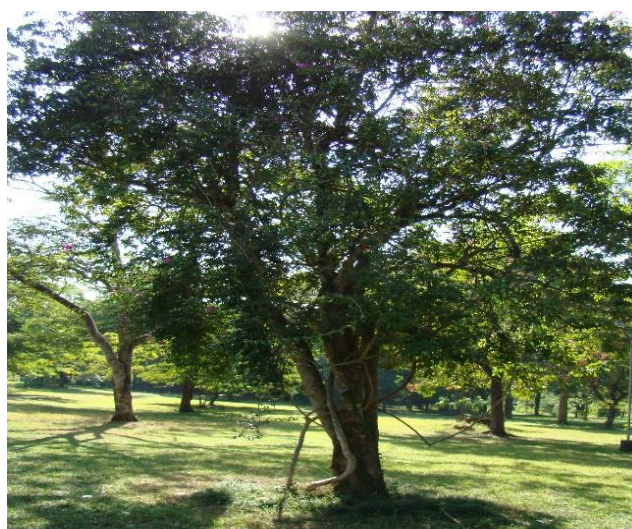
#### Chemical constituents:

Bark contains hentriacontane,  $\beta$ -sitosterol, sucrose, tannins, tramontane, dalbergichromene lupeol, acetyloleanolic acid,  $\gamma$ -sitosterol, (*R*)-latifolin, (*R*)-4-methoxydalbergione, methyl dalbergin and dalbergin. Heartwood contains (*R*)-dalbergione,  $\beta$ -sitosterol 2,4,6-trimethoxy acetophenone, dalbergin, latifolin, dalbergione, dalatinone, liquiritigenin, a phenanthrene-1,4-quinone and latinone.<sup>13,14</sup>

The dermatitis causing allergens, methoxydalbergione and methoxydalbergione are present in wood. Seeds contain 4-phenylcoumarin, sisafolin and retinoid, dalbin and OH-retinoid, dalbinol. A new dihydroflavonol glycoside, aromadendrin-4' - O-  $\beta$ -D-xylopyranoside, has been isolated from the aerial parts (Asoka, 1992; Rastogi & Malhotra, 1990; Ghani, 2003).



**B-sitosterol**



**fig. 1: Dalbergia latifolia tree**

#### 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, All other reagents and chemicals used in studies were of analytical grade.

## II. ANIMALS AND METHODS

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°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 (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.

### 1. Mice lethality test:

Swiss albino mice were treated with methanolic extract of Bark of *Dalbergia latifolia* or vehicle orally for 21 days. On the 7<sup>th</sup> and 17<sup>th</sup> day of the treatment, the animals were immunized with haemorrhagic septicaemia vaccine (HS vaccine). On the 21<sup>st</sup> day, the animals were challenged subcutaneously with 0.2 ml of lethal dose (25 x LD<sub>50</sub>) of *Pasteurella multocida* (bovine origin) containing 10<sup>7</sup> cells per ml. The animals were observed for a period of 72 hr. and the mortality ratio was determined using the formula.

Mortality ratio: Number of animal's dead/Total number of animals

**Table No:-1: Table showing Effect of methanolic of *Dalbergia latifolia* (100&200 mg/kg. po /day/5days) on mice lethality test in Swiss albino mice. N=6.**

Group	TREATMENT	Day-1 No of dead animals	Day-2 No of dead animals	Day-3 No of dead animals	MORTALITY RATIO
I	Distilled water (1 ml /Kg.po/day/10days) + Pasteurellamultocida(0.2ml/single dose)	3	3	-	100%
II	Levamisole (2.5 mg/Kg.po/day/10days) + Hemorrhagic septicemic vaccine(0.1 ml) + Pasteurella multocida(0.2ml/single dose)	-	1	1	33.33%
III	Bark of DL (100 mg /Kg.po/day/10days) + Hemorrhagic septicemic vaccine(0.1 ml) + Pasteurella multocida(0.2ml/single dose)	-	1	2	50%
IV	Bark of DL (200 mg /Kg.po/day/10days) + Hemorrhagic septicemic vaccine(0.1 ml) + Pasteurella multocida (0.2ml/single dose)	-	1	1	33.3%
V	Hemorrhagic septicemic vaccine(0.1 ml) + Pasteurella multocida(0.2ml/single dose)	-	3	2	83.33%

**2. Carbon clearance test:**

Swiss albino mice were treated with the methanolic extract of Bark of *Dalbergia latifolia* or vehicle orally for 5 days. After 48 hr of the last dose of the drug, animals were injected 0.1 ml of Indian ink via the tail vein. Blood samples were withdrawn at 0 and 15 min after injection. A 50 µl blood sample was mixed with 4 ml of 0.1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm. The phagocytic index K was calculated using the following equation:

$$K = (\text{Log}_e \text{OD1} - \text{Log}_e \text{OD2}) / 15$$

Where, OD1 and OD2 are the optical densities at 0 and 15 min respectively.

- **Effect of methanolic extract of *Dalbergia latifolia* (100&200 mg/Kg, po/day/5days) on Phagocytic Index in Swiss albino mice.**

Administration of Levamisole (2.5 mg /Kg, po/day / 5 days) significantly ( $P < 0.05$ ) increased Phagocytic Index when compared to control group.

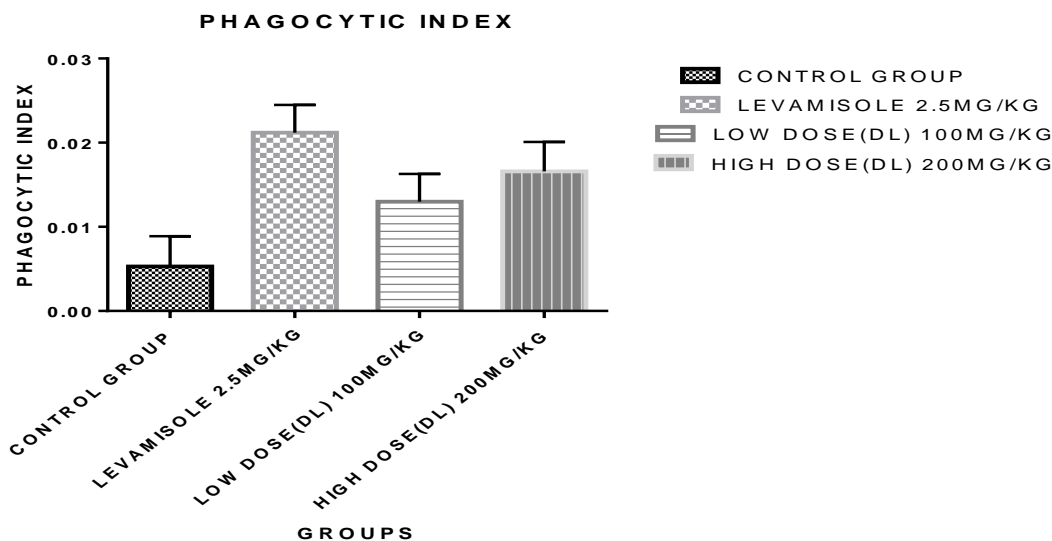
Administration of methanolic extract of Bark of DL (100mg /Kg, po /day /5days) significantly ( $P < 0.05$ ) increased Phagocytic Index when compared to control group.

Administration of methanolic extract of Bark of DL (200 mg /Kg, po /day / 5days) significantly ( $P < 0.05$ ) increased Phagocytic Index when compared to control group.

**Table:- 2: Table showing Effect of methanolic extract of *Dalbergia latifolia* (100&200 mg/kg. po /day/5days) on Phagocytic Index in Swiss albino mice.**

Group	TREATMENT	PHAGOCYtic INDEX
I	Vehicle (1 ml /Kg,po/day/5days)	0.0053 ± 0.0036
II	Levamisole (2.5 mg/Kg,po/day/5days)	0.0212 ± 0.0033*
III	Bark of DL (100 mg /Kg,po/day/5days )	0.0130 ± 0.0033*
IV	Bark of DL (200 mg /Kg,po/day/5days )	0.0166 ± 0.0035*

Values are expressed as mean ± SEM. n=6 \* $P < 0.05$  when compared to control.



**Fig- Effect of methanolic extract of *Dalbergia latifolia* on phagocytic index**

### III. CONCLUSION AND DISCUSSION

An immunomodulator is any substance that helps to regulate the immune system. This “regulation” is a normalization process, so that an immunomodulator helps to optimize immune response.

The results of the present study show that the Bark of *Dalbergia latifolia* affects both the cell mediated and humoral immunity.

Bark of *Dalbergia latifolia* increased the levels of serum Immunoglobulin level and prevented the mortality induced by bovine *Pasteurella multocida* in mice. On the other hand, it showed significant increase in the phagocytic index in carbon clearance assay.

Hence, it was concluded that Bark of *Dalbergia latifolia* increases both humoral immunity and cell mediated immunity.

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