

## Research Article



## Evaluation of Immunomodulatory Activity of Aerial Parts of *Delonix regia* by *in-vitro* and *in-vivo* Methods in Albino Wistar Rats: A Research

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### ABSTRACT

The aim of present study was to evaluate the immunomodulatory activity of methanolic extract of aerial parts of *Delonix regia* (Gulmohar) in experimental animals. Significant immunomodulatory potential was assessed by various In-Vitro method like T-Cell population and In-Vivo methods such as Delayed hypersensitivity reaction, carbon clearance test and neutrophil adhesion tests at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg. Various doses of methanolic extract of aerial parts of *Delonix regia* showed increase in phagocytic index in carbon clearance test and neutrophil adhesion. It also showed significant potential for increase in paw volume in case of DTH test. For T-Cell population methanolic extract of aerial parts of *Delonix regia* had significant increase in lymphocyte count and rosettes count as compared with control group. Methanolic extract of aerial parts of *Delonix regia* at dose of 400 mg/kg has showed results near to the group of levamisole treated group. Thus, methanolic extract of aerial parts of *Delonix regia* possesses immunostimulating activity by humoral and cell-mediated mechanism.

**Keywords:** Immunomodulator, *Delonix regia*, Carbon clearance, Neutrophil adhesion, T-Cell population.

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### INTRODUCTION

The immune system is a highly effective network of cellular components and substances that was created to protect the body's integrity from external shocks. Its proper operation and balance are crucial to prevent the formation of a wide range of illnesses. The difficult process of anticipating a disease involves selecting from a few visible symptoms and selecting a course of treatment based on its likelihood of being beneficial <sup>1</sup>.

An immunomodulation is a therapeutic approach in which there is auto regulating process of defense system and immunomodulators becomes viable adjunct to establish therapeutic modalities <sup>2</sup>. Immunomodulators are used to treat conditions such as multiple sclerosis, hereditary angioedema, rheumatoid arthritis, and Cryopyrin-associated periodic syndromes, Acute lymphatic leukemia, Crohn's disease, Ulcerative colitis, Pericarditis, Kidney transplant rejection, Capillary leakage syndrome, Familial cold auto-inflammatory syndrome and slow down the progression of the disease.<sup>3</sup> Cancer immunotherapy has dramatically increased patient quality of life and survival. Immunotherapy has solidified its position as a new pillar of cancer treatment. <sup>4</sup>

Many immunomodulators in the clinical use are cytotoxic drugs with significant adverse effects. To overcome toxicity and existing available cytotoxic drugs there is need for new immunomodulatory medications. Traditional medicines are the oldest approach for treating and managing any illness without causing severe or minor adverse effects. Further the search for chemicals of plant origin as fresh leads for creation of potent and safe immunomodulators is receiving a lot of attention.<sup>5</sup> Ayurveda, India's traditional medical system, describes some herbs that boost the immune system of the host. As a result, the ability of various plant extracts, or active fractions, to modulate immune response, has been studied. <sup>6</sup>

Furthermore, physiologically active chemicals derived from natural sources have long piqued researchers' curiosity when it comes to fighting infectious diseases or improving immune function. Numerous plant-based principles have been identified with potential immunomodulatory action, which can both explain and support their historical use in traditional medicine and serve as the foundation for future research. <sup>7, 8</sup> The immune system has been shown to be modulated by a number of plant-derived substances, including polysaccharides, lectins, peptides, flavonoids, alkaloids, and tannins. <sup>9,10</sup>

Gold Mohar [*Delonix regia* (Bojer ex Hook.) Raf.] Is a flowering plant that belongs to the *Fabaceae* family. It goes by the names Royal Poinciana, Flamboyant, and Flame tree. Gold Mohar is a medicinal and biological plant with numerous benefits. Chemical components in *Delonix regia* include  $\beta$ -sitosterol, quercetin, lupeol, vitamin E, gallic



acid, properlargonidin, alanine, valine, tyrosine, and Rhamnose may be the cause of the advantages.<sup>9</sup> This phytochemicals have reported for their anti-oxidant, anti-inflammatory and immunomodulatory activities. *Delonix regia* has been employed in folk medicine systems of several cultures for a variety of ailments, including rheumatism, arthritis, hemiplegia, leucorrhoea, and constipation. Plant of *Delonix regia* shows various pharmacological activities like anti-oxidant, anti-inflammatory, hepatoprotective and diuretics etc. The DPPH radical scavenging assay technique was used to measure the antioxidant activity. The activity largely due to flavonoid thus it removes free radicals and other reactive species. Hence the aerial parts of plant *Delonix regia* was used for the present research work.<sup>11, 12</sup> Present study is an attempt to find out the immunomodulatory activity of aerial parts of *Delonix regia* in animal models.

## MATERIALS AND METHODS

### Collection and extract preparation of plant material

The fresh stem, leaves and seeds of *Delonix regia* were collected from sangli city, Maharashtra. The plant was then authenticated by Mr. Wadmare of the Department of botany Kasturba Walchand College, Sangli, Maharashtra. The plant materials were cleaned, dried under shade and grinded to form powder. The powdered plant material (100 g) was then subjected for extraction with methanol using soxhlet apparatus. Then the extract was stored in air tight container.

### Animals used

In case of experimental work each animal of either male or non-pregnant female albino rat of wistar strain weighing between 160 to 210 grams were used. . The institutional animal ethics committee accepted the experimental methodology (IAEC/ABCP/02/2021-22), and the care of laboratory animals was handled in accordance with CPCSEA guidelines from the Indian Ministry of Forests and Environment.

### Chemicals and reagents

Leishmann's stain was procured from labine lab, Pune. Anesthetic ether was purchased from research lab fine chem industries Mumbai. Standard levamisole was brought from Johnson and Johnson Lmt.

### Preliminary qualitative screening of phytochemicals

The methanolic extract of aerial parts of *Delonix regia* were assessed for presence of phytochemicals by using various standard procedures.

### Dose Selection of Drug

For the dose selection of *Delonix regia* plant extract, it is the need to observe and know the toxicity level of extract in accordance with OECD guidelines. Up to a dosing level of 5000 mg/kg of body weight, *Delonix regia* was considered safe. No fatality or adverse effects were discovered, Up until the conclusion of the study period

when the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg were given. This doses already reported to safe in research papers.

### Sheep red blood cells (SRBCs) suspension

Alsever's solution (1:1) containing fresh sheep blood (taken from a nearby local market of sangli) was refrigerated. Centrifuging at 2000 rpm for 10 minutes and washing 4–5 times with physiological saline were used to prepare SRBC for vaccination. The cleaned SRBCs were then suspended at the required concentration in buffered saline.

### Experimental protocol

The methanolic extract of aerial parts of *Delonix regia* was dissolved in distilled water and doses selected at 100 mg/kg, 200 mg/kg and 400 mg/kg body weight.

Group I served as control and was given water as vehicle only.

Group II received standard dose of levamisole at 50 mg/kg.

Group III received 100 mg/kg methanolic extract of aerial parts of *Delonix regia*.

Group IV received 200 mg/kg methanolic extract of aerial parts of *Delonix regia*.

Group V received 400 mg/kg methanolic extract of aerial parts of *Delonix regia*.

MEAP- methanolic extract of aerial parts

### Delayed type hypersensitivity test<sup>13, 14</sup>

Animals were given continuous doses of plant extract up to 13 days. On 14<sup>th</sup> day, all animals in five groups were immunized by injecting 0.1 ml of 20% SRBCs in normal saline. On 21<sup>th</sup> day, all animals were re-challenged with 0.03 ml of 1% SRBCs in subplanter region of right hind paw. Foot pad reaction was assessed on 22<sup>nd</sup> day, which is due to hypersensitivity reaction results in oedema. Thickness was measured by vernier caliper/digital plethysmometer.

### Carbon clearance test<sup>13, 14</sup>

Animals were given dose of extract up to 10 days. On the 10<sup>th</sup> day, all the animals in each group were administered with 0.1 ml of carbon ink suspension through the tail vein as per the body weight. The blood sample was withdrawn from the retro-orbital plexus under mild ether anesthesia at 0 and 15 min quickly after injecting carbon suspension. Then blood sample (25 ml) was lysed with 2 ml of 0.1% sodium carbonate. Absorbance was measured spectrophotometrically at 675 nm to determine optical densities.

The phagocytic activity was measured by formula as per following,

$$k = \frac{\log OD1 - \log OD2}{t2 - t1}$$

Where,



OD<sub>1</sub>- optical density at time (t<sub>1</sub>) 0 min after blood withdrawn from tail vein of rat.

OD<sub>2</sub>- optical density at time (t<sub>2</sub>) 15 min after blood withdrawn from tail vein of rat.

#### Neutrophil Adhesion test<sup>13,14</sup>

At the end of treatment of 16<sup>th</sup> day, blood sample was collected through retro-orbital plexus under mild anesthesia. Blood was collected in the heparinized blood vials and then analyzed for total leukocyte count (TLC) and differential leukocyte count. After initial blood count, blood samples were treated with nylon fiber (80mg/kg) which is firstly sterilized by 95% of alcohol for 15 min at 37°C. TLC and DLC of incubated sample were carried out. Further the product of TLC and % neutrophil was considered as Neutrophil Index (NI) and neutrophil adhesion was calculated.

The formula for calculating neutrophil adhesion as follows,

$$\% \text{ Neutrophil Adhesion} = \frac{NIU - NIT}{NIU} \times 100$$

Where,

NI<sub>U</sub>- Neutrophil Index of untreated blood sample.

NI<sub>T</sub>- Neutrophil Index of Nylon Fibre treated blood sample.

#### T-Cell population test<sup>13,14</sup>

Antigen challenge- before the one day of treatment, all the animals from groups were sensitized with 0.1 ml of SRBCs which contains 1 x 10<sup>8</sup> cells, i.p. On the last day of treatment, i.e. 11<sup>th</sup> day blood sample was collected in heparinized vials and anticoagulated with Alsever's Solution in separate test tubes. Then the sample of blood contains in test tubes were kept in sloping position (45°)

for about 1 hours. Then the RBCs were allowed to settle at the bottom and supernatant was taken out from each test tube by using micropipette lymphocytes. After that, 50 ml of blood lymphocyte solution and 50 ml of SRBCs were combined and incubated in a test tube. The resulting suspension was centrifuged for 5 minutes at 200 rpm before being chilled for 2 hours at 40°C. One drop of the cell suspension was applied to a glass slide after the supernatant fluid had been removed. Total lymphocytes were counted, and the number of rosettes was calculated as each lymphocyte that bound to three or more erythrocytes, or RBCs.

#### Statistical Analysis

Mean Value ± SEM was used to express the test's outcome. The one way analysis of variance (ANOVA) technique was used to estimate the variation in a collection of data. The individual comparison of group mean value were done by Dunnet's Test. Statistics were considered significant when the P value < 0.05.

## RESULTS AND DISCUSSION

### Delayed type Hypersensitivity

Delayed hypersensitivity test is a type IV hypersensitivity reaction which was used to measure the skin reaction after inoculation of the antigen. This antigen triggers specific memory T cells that in turn responsible for inflammation process. Further, it helps to improve lytic enzymes concentration as well as phagocytic activity for more efficient microbial destruction. Thus, previously immunized rats with given doses of methanolic extract of aerial parts of *Delonix regia* showed increase in paw volume as compared to the control. (Table 1)

**Table 1:** Results of DTH (increase in paw edema)

Sr.no	Groups	treatments	Dose and route of administration	Mean difference in paw edema in (mm) X (mean ± SEM)	Percent increase in paw edema
1	I	Control	10 ml/kg (p.o)	2.163±0.02716	100%
2	II	Standard (levamisole)	50 mg/kg (p.o)	4.277±0.02985****	197.73%
3	III	MEAP of delonix regia	100 mg/kg (p.o)	2.260±0.03890 <sup>ns</sup>	104.48%
4	IV	MEAP of delonix regia	200 mg/kg (p.o)	3.703±0.02848****	117.19%
5	V	MEAP of delonix regia	400 mg/kg (p.o)	4.170±0.02066****	192.78%

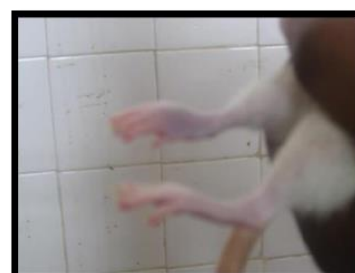
### DTH response of Paw oedema observed in various groups challenged with SRBCs



A. Control animal



B. Delonix regia 400 mg/kg



C. Levamisole 50 mg/kg

**Table 2:** Results of Carbon Clearance Test

Groups	treatments	Dose and route of administration	Phagocytic Index X= (Mean ± SEM)	Increase in phagocytic index
I	Control	10 ml/kg (p.o)	0.02337±0.001618	100%
II	Standard (levamisole)	50 mg/kg (p.o)	0.06042±0.001765****	258.53%
III	MEAP of <i>Delonix regia</i>	100 mg/kg (p.o)	0.02497±0.002014 <sup>ns</sup>	106.84%
IV	MEAP of <i>Delonix regia</i>	200 mg/kg (p.o)	0.03428±0.001677**	106.84%
V	MEAP of <i>Delonix regia</i>	400 mg/kg (p.o)	0.05128±0.002535****	219.42%

**Carbon clearance test**

Reticuloendothelial system (RES) was mainly responsible to causes phagocytosis. Exogenously administered antigen like colloidal carbon ink preparation, are recognized as foreign invaders and amputated by RES through this process of phagocytosis. Increase in particle remove activity from blood stream indicated increase in phagocytic activity. Further, the relationship between phagocytosis and the phagocytic index is a measurement of carbon clearance test.

The phagocytic index increased noticeably in *Delonix regia* treated rats, and it is hypothesised that this might be because the animals had previously received treatment that predisposed them towards reticuloendothelial system to become more active. 400mg/kg methanolic extract of aerial parts of *Delonix regia* plant increases phagocytic index close to results obtained by standard levamisol which is the indicator of increased in vivo phagocytic activity and granulopoetic system competency in removing foreign particles, which is a sign of increased immune response to foreign particles or antigens. (Table 2)

**Neutrophil adhesion test**

Neutrophils are the non-dividable cells responsible for various immune responses such as exocytosis, phagocytosis, chemotaxis, both intracellular and extracellular killing. Significant adherence is necessary for blood stream neutrophil marginalisation, and this adhesion is started by contacts between the neutrophils' b2 integrins. The migration of neutrophil granulocytes into the blood vessels and the accumulation of neutrophils at the site of inflammation are both indicated by neutrophils adhering to nylon fibres. Methanolic extract of aerial parts of *Delonix regia* at low dose of 100 mg/kg and at high dose of 400 mg/kg and both showed a significant increase in neutrophil adherence to nylon fibres. Which indicated by the b2 integrins that are found on the surface of neutrophils and allow them to cling securely to the nylon fibres, may have been upregulated. (Table 3)

**T-Cell population**

The rise in lymphocyte and rosette formation in the T cell population test shows how *Delonix regia*, extract at different doses, affects cell-mediated immunity. The extract may stimulate CD4 and CD8 cells, which can have a major impact on T-cell immunity response and T-cell mechanism. (Table 4 and 5)

**Table 3:** Results of Neutrophil Adhesion Test

Groups	treatments	Dose and route of administration	% Neutrophil Adhesion Mean (Mean ± SEM)
I	Control	10 ml/kg (p.o)	30.84±1.001
II	Standard (levamisole)	50 mg/kg (p.o)	64.04±1.017****
III	MEAP of <i>Delonix regia</i>	100 mg/kg (p.o)	39.11±0.8227****
IV	MEAP of <i>Delonix regia</i>	200 mg/kg (p.o)	48.29±0.7339****
V	MEAP of <i>Delonix regia</i>	400 mg/kg (p.o)	57.92±0.5735****

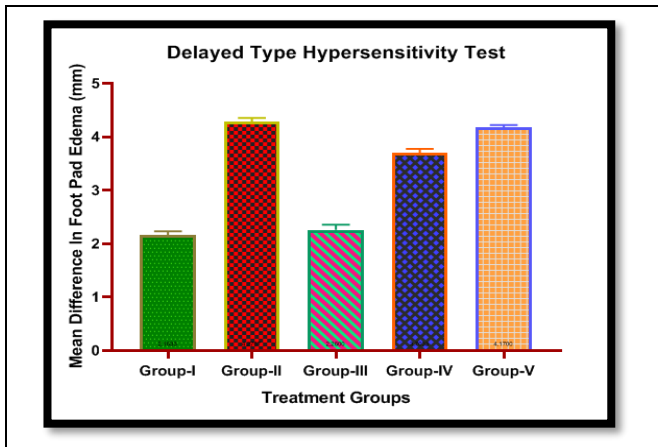
**Table 4:** Results of total lymphocyte count

Groups	treatments	Dose and route of administration	Total lymphocyte count in per mm <sup>3</sup> X=(Mean±SEM)	Percent increase in total lymphocyte count
I	Control	10 ml/kg (p.o)	642.8±44.68	100%
II	Standard (levamisole)	50 mg/kg (p.o)	2147±52.04****	334%
III	MEAP of <i>delonix regia</i>	100 mg/kg (p.o)	748.2±78.23 <sup>ns</sup>	116.36%
IV	MEAP of <i>delonix regia</i>	200 mg/kg (p.o)	1518±37.10****	236.15%
V	MEAP of <i>delonix regia</i>	400 mg/kg (p.o)	1983±63.17****	308.49%

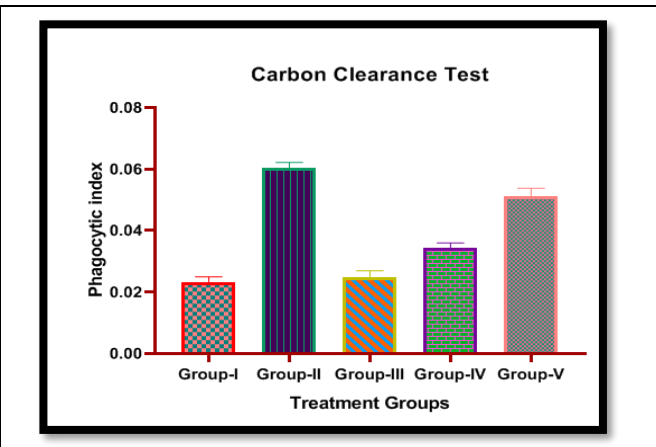


**Table 5:** Results of Rosettes count for T-Cell population

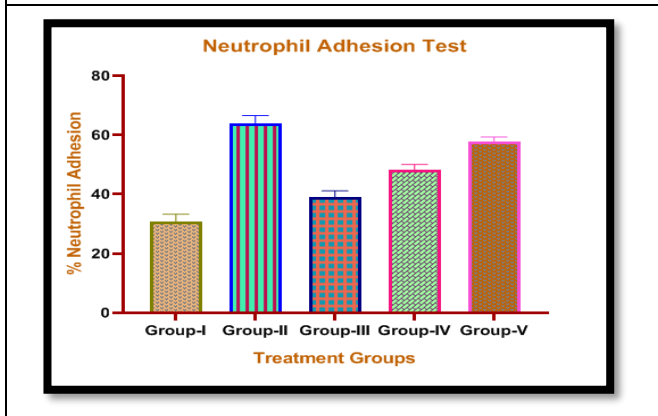
Groups	treatments	Dose and route of administration	Number of rosettes X (Mean± SEM)	Percent increase in rosettes formation
I	Control	10 ml/kg (p.o)	8.667±0.7601	100%
II	Standard (levamisole)	50 mg/kg (p.o)	28.00±1.211****	323.06%
III	MEAP of <i>Delonix regia</i>	100 mg/kg (p.o)	9.500±0.4282 <sup>ns</sup>	109.615%
IV	MEAP of <i>Delonix regia</i>	200 mg/kg (p.o)	17.17±1.276****	198.10%
V	MEAP of <i>Delonix regia</i>	400 mg/kg (p.o)	22.33±1.520****	257.64%



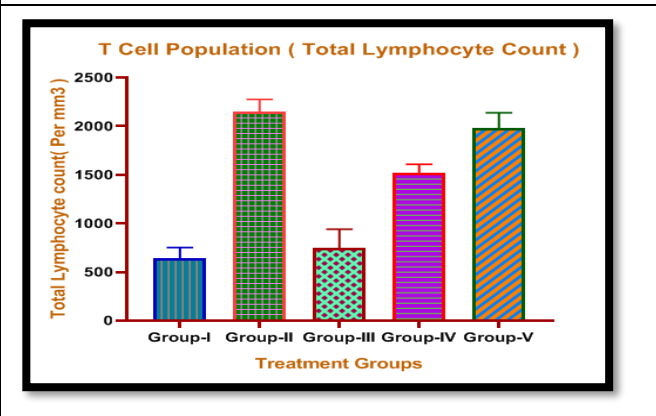
**Figure 1:** Graphical Representation of *Delonix regia* extract for DTH Test



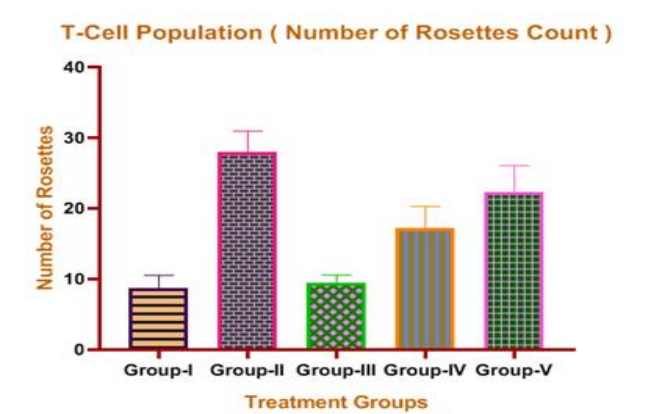
**Figure 2:** Graphical Representation of *Delonix regia* extract for Carbon clearance test



**Figure 3:** Graphical Representation of *Delonix regia* - extract for Neutrophil Adhesion Test



**Figure 3:** Graphical Representation of *Delonix regia* - extract for lymphocyte count



**Figure 4:** Graphical Representation of *Delonix regia* for Number of rosettes Count

**CONCLUSION**

Results indicates that cellular & humoral immune reponses were aroused by treatment of animal with methanolic extract of aerial parts of *Delonix-regia*. Based on the findings from the study, the doses of methanolic extracts of aerial parts of *Delonix-regia* showed significant immunostimulant effects in wistar rats & which can be used to uplift the Immune system in the infectious diseases condition.

The overall order of immunomodulatory activity,  
 Levamisole> Methanolic extract of *Delonix regia* (400mg/kg)  
 > Methanolic extract of *Delonix regia* (200mg/kg) >  
 Methanolic extract of *Delonix regia* (100mg/kg)

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