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RESEARCH ARTICLE

# *In Vivo* Study of Immunomodulatory Effect of *Gmelina arborea* Roxb.

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## ABSTRACT

To study immunomodulatory activity of hydroalcoholic plant extract of *Gmelina arborea* Roxb. The test animals chosen for the present experiment were Wistar albino rats. The tests carried out were haemagglutination inhibition, delayed type hypersensitivity test, complete blood counts and histopathology (Liver and Spleen). The dried plant powder extract of *Gmelina arborea* Roxb. was evaluated for immunosuppressive activity using Cyclophosphamide as an immunosuppressive drug and for immunostimulant activity using Septilin as an immunostimulant drug. Humoral immune response was evaluated by withdrawing blood from immunized wistar albino rats for haemagglutination inhibition test. Cell mediated immune response was studied using paw edema test conducted on immunized wistar albino rats. The blood cell counts were evaluated for generalized study of effect of the plant drugs of *Gmelina arborea* Roxb. Histopathological examination of liver and spleen were evaluated for the effect of plant drug of *Gmelina arborea* Roxb. on liver and spleen. The results obtained indicates that the plant *Gmelina arborea* Roxb. Possess immunostimulant activity *in vivo*.

## KEYWORDS

Immunomodulatory activity, *Gmelina arborea* Roxb., Wistar albino rats, Humoral immune, Cell mediated immune, Blood cell counts, Histopathology

## INTRODUCTION

A large number of plants included to promote the physical mental and defense mechanism in to the body. In other hand a large number of medicinal plants included in Rasayanas have been claimed to possess immunomodulatory activities. Medicinal plants which are used as immunomodulatory effect to provide alternative potential to conventional chemotherapy for a variety of diseases, especially in relation to host defense mechanism. The use of plant product like polysaccharides, lectins, peptides, flavonoids and tannins has been the immune response or immune system in various *in-vitro* modals[1]. The immune system is a part of body to detect the pathogen by using a specific receptor to produce immediately response by the activation of immune components cells, cytokines, chemokines and also release of inflammatory mediator. In the innate immune the nature killer cell plays an important role to the defiance against virus-infected and malignant cell to destroy the abnormal cell[2].

*Gmelina arborea Roxb.* (Family: Verbenaceae) is listed in Ayurveda as rasayana plant[3]. Hence, in the present research work, *in vivo* immunomodulatory activity of hydroalcoholic extract of plant powder of *Gmelina arborea* Roxb. was evaluated.

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## MATERIAL AND METHODS

### Plant Material

*Gmelina arborea* Roxb. was collected from KeshavShrishti, Maharastra. The plant material

was authenticated from Agharkar Research Institute, Pune, India (Auth.15-193).

The plant material was washed with water to remove soil particles, dried in shade, finely powdered and then sieved through BSS mesh size 85 and stored in an airtight container at room temperature (25 ± 2° C).

**Preparation of Sample Solution**

About 2.0g of dried powder of whole plant of *Gmelina arborea* Roxb. was accurately weighed and transferred to a 100 mL stoppered conical flask. 50.0 mL of ethanol : water (1:1 v/v) was added to it and the flask was sonicated in an ultrasonic bath for 15 minutes. The flask was then shaken at 50 rpm, on a conical flask shaker overnight at room temperature (25 ± 2°C). Sample was filtered through Whatman filter paper no.1 of pore size 11 µm. The filtrate was then finally filtered using 0.45 µm nylon filters (Millipore), collected in a beaker and then evaporated to dryness on hot water bath. The final volume was then made up to 10mL with distilled water in a volumetric flask.

**Chemicals**

The drug, Cyclophosphamide monohydrate (purity 99.5%) was procured from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany). and Septilin (Himalaya Drug Company, Bangalore) was procured from a local chemist shop.

Sheep RBCs were procured from Sheep Farm, Bombay Veterinary College, Goregaon, Mumbai and were used as the antigen for the heamagglutination test. Sheep RBCs were collected in Alsever's solution, washed in pyrogen free, sterile, 0.9% normal saline, and were used (0.5 x $10^9$ cells per ml per 100gm body weight of rat) intraperitoneally for immunization.

**Experimental Protocols**

The study was approved by the Institutional Animal Ethics committee, Mumbai Veterinary College, Parel, Mumbai-12. Experimental animals were handled according to the University and Legalization, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India; vide approval number MVC/IAEC/08/2014.

**Experimental Animals**

Wistar strain of Male and female Albino Rats aged about 3-4 weeks, approximately weighing between 150-200gms were used in the present study.

Wistar albino rats were divided into five groups, each group consisting of four males and four females.

Group I received normal feed for all 14 days. Rest all groups were immunized with antigen, Sheep RBC on $10^{th}$ and $14^{th}$ day.

Group II received only sheep RBC

Group III received cyclophosphamide (50mg/ kg bdwt) on $1^{st}$ and $14^{th}$ day intravenously.

Group IV received Septilin (500 mg/kg bdwt) dose on all 14 days orally.

Group V received *Gmelina arborea* Roxb. extract on all 14 days orally.

**Immunomodulatory Study Tests**

***Determination of Humoral Immune Response by Haemagglutination Inhibition (HI)***

The animals were immunized by injecting 100µL of 1 X $10^8$ SRBCs/mL intraperitoneally (i.p.) on day 0 and day 7. Blood samples were collected in eppendorf tubes from individual animals of all the groups by retrorbital vein puncture on $15^{th}$ day. The blood samples were centrifuged, the serum was separated. Initially, 50 µL of chilled normal saline solution was transferred to all the 96 wells of U-bottom microtiter plates. Then, 50 µL of serum was placed in the first well of the same 96 well U-bottom microtiter plate and mixed. 50 µL from first well was then withdrawn and added to second well. Again 50 µL of mixture was withdrawn from the second well and transferred to third well. Similar procedure was done till tenth well. Finally 50 µL from the tenth well was withdrawn and discarded. Haemaglutination titres were then performed. The reciprocal of the highest dilution of the

serum that completely inhibited agglutination (button formation) of the SRBC was taken as HI titre. (Figure 1)

***Determination of Cell Mediated Immune Response (Delayed Type Hyper Sensitivity Test)***

The animals of all groups were immunized by 0.1 mL of SRBC suspension containing 1x $10^8$ cells, intraperitoneally, on day 0.On $14^{th}$ day the thickness of right hind foot pad of each rat was measured using Vernier caliper (Mitutoyo, Japan).Right foot pad of each rat was injected with 1 x $10^8$ SRBCs. The foot pad thickness was measured again after 48hr. after the challenge. (Figure 2)

***Determination of Complete Blood count***

Yaccua Tubes with rubber cap and outer caps containing sodium citrate (3.2%) as anticoagulant were used for collection of blood withdrawn by retro-orbital plexus.

The cell count observed in the blood was counted using Neubauer chamber (Brand GMBH, Wertheim, Germany), followed by microscopic examination of Wright-stained smears with 100 X objective.

Complete blood count was evaluated using Red blood counts, White blood counts, Neutrophil %, Eosinophil %, Lymphocyte %, Monocyte % and Platelet counts. (Table I)

***Histopathological Evaluation***

The organs, liver and spleen of the animals of respective groups were collected in 10% formalin solution on final day after sacrifice by cervical dislocation. The microtomes of the organs were prepared in wax. Staining was carried out with haematoxylin-eosin and the slides of liver and spleen were observed under light microscope (magnification at 100X). The results of these analyses were compared with that of control. (Table 2 and Figure 3).

## RESULTS

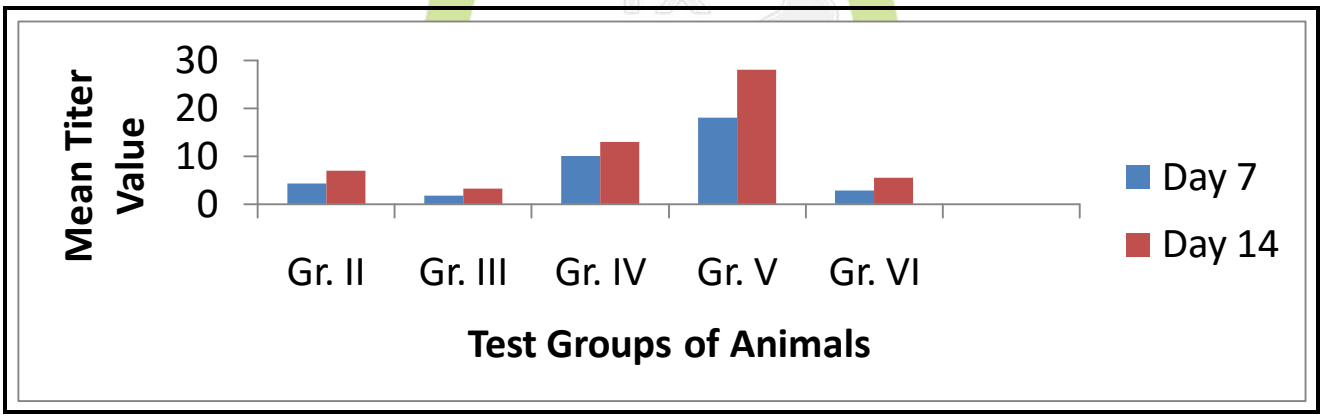



Figure 1: Graph of Mean haemagglutination inhibition titre values against different drug administration's to groups

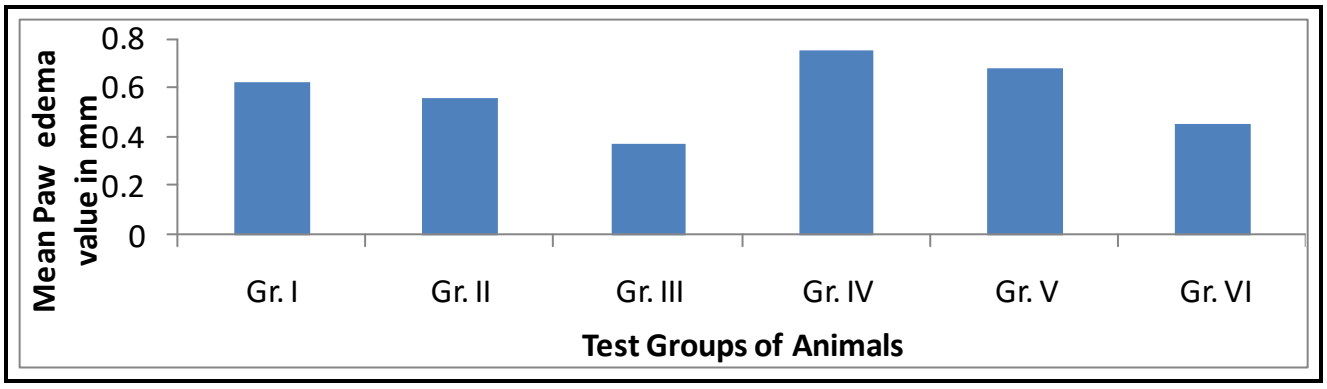



Figure 2: Graph of Mean values of reduction in foot pad thickness observed after 48hrs in delayed type hypersensitivity test against different drug administration's to groups

Table 1: Complete blood count results

| Parameters | Time | Groups | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | V |
| Hamagglutination titre values | Day 7 | - | 4.25±0.59 | 1.75±0.25 | 10.00±1.31 | 18.00±2.00 |
| | Day 14 | - | 7.00±0.65 | 3.25±0.37 | 13.00±1.46 | 28.00±2.62 |
| Delayed Type Hypersensitivity (Foot pad thickness) | After 48 hrs | 0.62±0.07 | 0.56±0.05 | 0.37±0.03 | 0.75±0.04 | 0.68±0.04 |
| Percent Delayed Type Hypersensitivity | After 48 hrs | 13.07±1.43 | 10.97±1.12 | 6.20±0.51 | 14.50±1.46 | 10.72±0.47 |
| Red Blood Counts | Day 0 | 6.83±0.12 | 6.68±0.13 | 6.54±0.14 | 8.22±0.08 | 8.00±0.13 |
| | Day 7 | 6.40±0.04 | 6.90±0.20 | 5.37±0.04 | 7.48±0.09 | 7.55±0.14 |
| | Day 15 | 7.84±0.07 | 7.79±0.08 | 6.05±0.13 | 7.88±0.05 | 7.77±0.07 |
| White Blood Counts | Day 0 | 11.18±0.74 | 10.36±0.99 | 9.69±0.49 | 12.24±0.32 | 10.36±0.33 |
| | Day 7 | 11.96±0.90 | 10.43±0.98 | 1.71±0.11 | 11.34±0.27 | 11.13±0.39 |
| | Day 15 | 16.8±0.75 | 13.2±0.31 | 2.23±0.15 | 13.0±0.40 | 12.2±0.34 |
| Neutrophil Count % | Day 0 | 22.5±1.84 | 19.3±0.80 | 21.9±1.23 | 14.3±0.70 | 17.25±1.41 |
| | Day 7 | 20.5±2.08 | 24.5±0.87 | 32.1±2.38 | 17.5±0.87 | 24.8±2.19 |
| | Day 15 | 45.1±4.59 | 44.0±3.08 | 22.5±1.16 | 39.4±0.66 | 12.2±0.60 |
| Eosinophil Count % | Day 0 | 0.36±0.32 | 0.50±0.33 | 0.25±0.16 | 0.50±0.19 | 0.25±0.16 |
| | Day 7 | 1.13±0.35 | 0.75±0.37 | 0.13±0.13 | 0.63±0.18 | 0.38±0.18 |
| | Day 15 | 0.25±0.16 | 0.25±0.16 | 0.38±0.18 | 0.38±0.18 | 0.50±0.19 |
| Lymphocyte Count % | Day 0 | 69.6±1.90 | 63.1±1.56 | 57.4±2.2 | 68.3±1.64 | 64.0±2.00 |
| | Day 7 | 77.3±2.14 | 73.0±1.04 | 69.6±1.88 | 84.6±0.94 | 73.9±2.14 |
| | Day 15 | 61.3±4.25 | 40.1±3.94 | 33.5±1.57 | 58.6±3.10 | 39.3±0.41 |
| Monocyte Count % | Day 0 | 0.38±0.18 | 0.38±0.18 | 0.25±0.16 | 0.50±0.33 | 0.50±0.27 |
| | Day 7 | 0.63±0.18 | 0.50±0.19 | 0.25±0.16 | 0.75±0.31 | 0.75±0.25 |
| | Day 15 | 0.63±0.18 | 0.25±0.16 | 0.13±0.13 | 0.75±0.16 | 0.63±0.18 |
| Platelet Counts | Day 0 | 7.34±0.23 | 7.18±0.33 | 7.43±0.25 | 7.09±0.23 | 7.05±0.12 |
| | Day 7 | 5.00±0.16 | 5.73±0.15 | 3.08±0.10 | 5.74±0.22 | 5.83±0.06 |
| | Day 15 | 7.54±0.24 | 7.15±0.68 | 6.8±0.20 | 8.3±0.42 | 8.7±0.13 |

*Note: Values are expressed as the mean ± SEM; (n = 8) for each group. SEM - Standard Error Mean

Table 2: Histopathology Results

| Testing | Histopathology of Liver | Histopathology of Spleen |
|---|---|---|
| **Group I** | Mild degree diffuse granular degeneration | No abnormalities were detected |
| **Group II** | Mild to moderate degree diffuse granular degeneration | No abnormalities were detected |
| **Group III** | Mild degree diffuse granular degeneration | No abnormalities were detected |
| **Group IV** | Mild degree diffuse granular degeneration | No abnormalities were detected |
| **Group V** | Moderate degree diffuse granular degeneration | No abnormalities were detected |

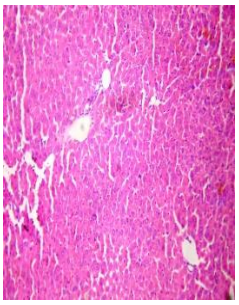
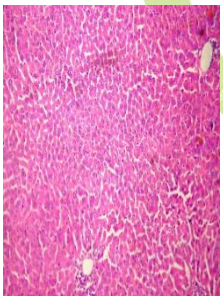
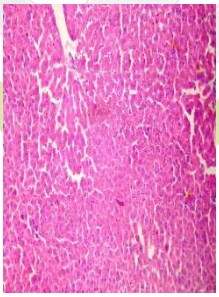
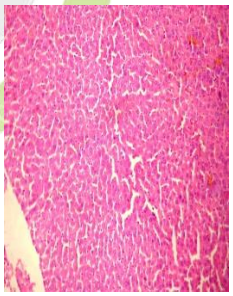
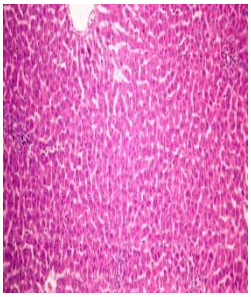
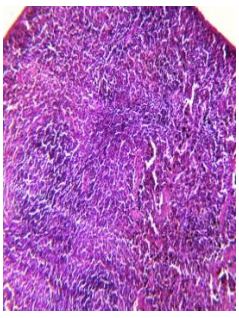
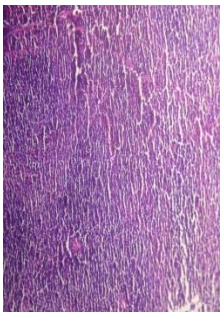
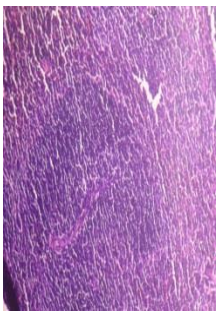
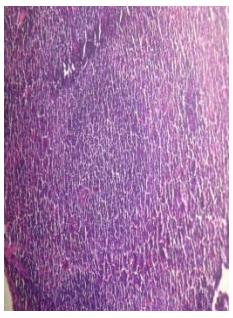
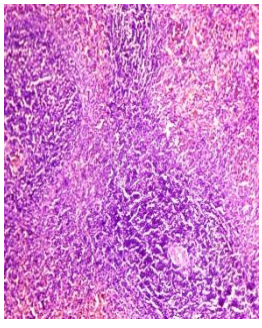
| Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|
| Histology slides of Liver of Animals | | | | |
|  |  |  |  |  |
| Histology slides of Spleen of Animals | | | | |
|  |  |  |  |  |

Figure 3

## DISCUSSION

The study was designed to evaluate the effectiveness of the hydroalcoholic extract of *Gmelina arborea* Roxb. stated to have immunomodulating property[4]. The animal model chosen for the present study were wistar albino rats. Standard cyclophosphamide was used as an immunosuppressant due to its action of DNA-alkylation leading to cell death and leucopenia[5]. Septilin was used for immunostimulation, as it has been reported to develop resistance to infections[6]. The study was performed over a period of fourteen days. Wistar albino rats were immunized on zero day to generate an immune response. The immune response was then assessed by determining the humoral immunity, cell mediated immunity and blood cell counts. Histopathological examinations of liver and spleen were done.

Immunomodulatory study was carried out with tests suggested in literature[7]. The tests conducted for determining the *in vivo* immunomodulatory activity of *Gmelina arborea* Roxb. were haemagglutination test for determining humoral immune response and delayed type hypersensitivity test for determining cell mediated immune response. The complete blood cell counts and histopathological examination of liver and spleen were also evaluated.

Haemagglutination Inhibition test was performed using sheep RBCs as an antigen to assess effects of various treatments on humoral immune response. It was observed that haemagglutination titre values for hydroalcoholic extract of *Gmelina arborea* Roxb. showed improved humoral immune response as compared to group II (only antigen). The hydroalcoholic extract of *Gmelina arborea* Roxb. administered to group V showed an increase in humoral immunity response as compared to group IV, administered with septilin. This shows that hydroalcoholic extract of *Gmelina arborea* Roxb. shows immunostimulating activity and is more immunostimulant than a known immunostimulant, Septilin. Delayed type hypersensivity response was evaluated using sheep red blood cells as an antigen to assess effects of various treatments on cell mediated immune response. The test was performed by assessing paw edema size reduction. The hydro-alcoholic extract of *Gmelina arborea* Roxb. Administered to group V showed boosted cell mediated response for delayed type hypersensitivity as compared with group II (only antigen). Group IV administered with septilin showed boosted cell mediated response for delayed type hypersensitivity as compared with group V. This shows that hydroalcoholic extract of *Gmelina arborea* Roxb. Shows immunostimulating activity but is less immunostimulant than a known immunostimulant, Septilin.

Complete Blood Cell count was done to evaluate the counts of Red blood cells, White blood cells and Platelets. Counts of Red blood cells, White blood cells, Neutrophils, Eosinophils, Lymphocytes, Monocytes and Platelets were noted for significant changes. Hydro alcoholic extract of *Gmelina arborea* Roxb., administered to group V showed increased counts of Red blood cells, White blood cells, Neutrophils, Eosinophils, Lymphocytes, Monocytes and Platelets as compared to group II (only antigen) but this increase is less as compared to the increase observed in group IV, administered with septilin.

The histopathology of the liver revealed that hydro alcoholic extract of *Gmelina arborea* Roxb., administered to group V showed moderate degree diffuse granular degeneration in liver of test animals as compared with liver of test animals of group II (only antigen).

The histopathology of the spleen revealed that hydroalcoholic extract of *Gmelina arborea* Roxb., administered to group V showed no abnormalities in spleen of test animals as compared with spleen of test animals of group II (only antigen).

Therefore, after evaluation of all the above parameters, it was observed that hydroalcoholic extract of whole plant powder of *Gmelina arborea* Roxb., showed immunomodulating activity *in vivo*.

In a reported method[8], a similar kind of immunomodulatory study was done to assess the efficacy of hydro-alcoholic extract of flowers of *Hibiscus rosa sinensis* Linn. and ethanolic extracts of aerial parts of *Cleome gynandra* Linn. The study was done *in vivo*. The results showed immunostimulatory activity of *Hibiscus rosasinensis* Linn. and immunosuppressive activity of *Cleome gynandra* Linn.

In another reported method[9], a similar immunomodulatory study was done to assess the efficacy of saline extracts of leaves of *Aloe vera* Linn. The results showed that *Aloe vera* extract produces stimulatory effect on the humoral and cell mediated immune response in the albino mice.

However, as compared to the all the above methods, in the present study, additional parameters of Complete blood count and histopathological evaluation were done to find the efficacy of plants *Gmelina arborea* Linn., more effectively.

Thus, the literature survey revealed that these tests were not carried out for hydroalcoholic extract of *Gmelina arborea* Roxb., and in the present research work, immunomodulatory activity was proved with additional parameters of humoral immune response, cell mediated immune response, cell blood count and histopathological evaluation.

## CONCLUSION

The dried whole plant extract of *Gmelina arborea* Roxb. showed an increase in titre values, delayed type hypersensitivity response and complete blood count.

The histopathological examinations revealed changes in histology of liver and spleen supporting the above conclusion.

Hence, all of the above observations reveal modulating effect of *Gmelina arborea* Roxb. extracts. Thus, the plant *Gmelina arborea* Roxb. is found to possess immunostimulant activity.

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