



## PRELIMINARY IMMUNOMODULATORY ACTIVITIES OF AQUEOUS EXTRACT OF *MORUS ALBA* LINN

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### ABSTRACT

Aqueous leaf extract of *Morus alba* was evaluated for the immunomodulatory activity. Wistar rats were used as the specimen. The extract is tested for hypersensitivity and hemagglutination reaction using sheep red blood cells (SRBC) as the antigen. The *Morus alba* (200 & 400 mg/kg p.o) offers an increase in delayed type hypersensitivity reaction and the effect is comparable with that of the standard drug levamisole. It does not induce any significant alterations in antibody titer value. *Morus alba* however facilitates a considerable increase in total leukocyte, lymphocyte, neutrophil and eosinophil count. In comparison to 200 mg/kg dose, 400 mg/kg dose of *Morus alba* was found to induce a better immunomodulatory activity. It is inferred that *Morus alba* aqueous extract stimulates the innate or non-specific immune system in a dose dependant manner and does not stimulate the adaptive immune system in mediating immunomodulatory property.

**Key words:** Immunomodulation, Hemagglutination, *Morus alba*, Polymorphonuclear cells, Leukocytes, Sheep's red blood cells, Delayed type hypersensitivity reaction, Peroral.

### INTRODUCTION

Plants and animal products have been the basis of treatment for human diseases since ancient times. Ayurveda has been one of the traditional systems of medicine practiced in India<sup>1</sup>. It has been recognized that immunomodulation could provide an alternate to conventional chemotherapy in the treatment of various diseases. An improved understanding of the immune system has led to the development of new therapies to treat immune system mediated diseases<sup>2</sup>. Mulberry fruit juices have been used as brain tonic, diuretic; roots as anthelmintic, purgative and astringent<sup>3</sup>. The earlier report has indicated enhancement of neuroprotective activity of mulberry leaves<sup>4</sup>. *Morus alba* leaf extract has been found to produce nitric acid, prostaglandin E2 and cytokines in macrophages<sup>5</sup>. Further, a

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polysaccharide isolated from *Morus alba* root bark has been found to produce immunomodulatory property<sup>6</sup>. The details of inducement of immunomodulatory property of 1-deoxynojirimycin, which is a major constituent of *Morus alba*<sup>7</sup>, is not available. Hence, the aqueous extract of the leaves of *Morus alba* is being investigated in the present study for immunomodulatory activity using Wistar rats and sheep red blood cells (SRBC) model.

## EXPERIMENTAL

### Collection of plant materials

The dried leaves of *Morus alba* belonging to family Moraceae were obtained from Phytotek Extracts Pvt. Ltd., Bangalore, India and sufficiently authenticated.

### Preparation of extract

The powdered leaves of *Morus alba* (1 kg) were extracted by hot maceration process for one hour with sufficient quantity of distilled water. The filtered extract was subjected to freeze-drying and stored in a dessicator until use.

### Animals

After due permission from Institutional Animal Ethical Committee (Certificate No. 396 dated 16.11.2006) the experiments were carried out. Wistar rats were procured and maintained at an ambient temperature ranging between ( $25 \pm 5^{\circ}\text{C}$ ), with ( $55 \pm 10\%$ ) relative humidity and 12 hrs light/dark cycles for a period of one month. They were provided with access to standard pellet diet and water *ad libitum*.

### Preparation and administration of drugs

Levamisole (50 mg/kg) and freeze dried aqueous leaf extract of *Morus alba* (200 & 400 mg/kg) were dissolved in distilled water and administered orally for 14 days.

### Preparation of antigen

Sheep red blood cells were collected in Alsevers solution and washed with normal saline. The cells are adjusted to a concentration of 0.1 mL containing  $1 \times 10^8$  cells for immunization.

### Methods

#### Determination of delayed type hypersensitivity response (DTH)

The rats were immunized by injecting 0.1 mL of SRBCs suspension, containing 1 x

$10^8$  cells, intraperitoneally on the first day. On the eighth day, after immunization the thickness of the right hind footpad was measured using a standard vernier caliper. The rats were then challenged through an injection with  $1 \times 10^8$  SRBCs in the left hind footpad. The footpad thickness was measured again after 24 hours of challenge. The difference between the pre and post challenge footpad thickness, expressed in mean percentage increase, was considered as a measure of the DTH response<sup>8</sup>.

The result was calculated by the following formula:

$$\frac{\text{Left footpad challenged with antigen} - \text{Right footpad control}}{\text{Left footpad challenged with antigen}} \times 100$$

### **Humoral antibody titre**

The rats were immunized by injecting 0.1 mL of SRBCs suspension, containing  $1 \times 10^8$  cells, on the first day intraperitoneally. Blood samples were collected in centrifuge tubes from individual rats of all the groups by retro orbital vein puncture on the tenth day. The blood samples were centrifuged and the serum separated. Antibody levels were determined by the hemagglutination technique<sup>9</sup>.

Total leukocyte count (TLC) and differential leukocyte count (DLC) were determined following the standard procedure on the fourteenth day of the experiment.

### **Statistical analysis**

The data are expressed as Mean  $\pm$  SEM and subjected to one-way ANOVA, followed by a post hoc comparison using Dunnett's t test. The probability level more than 95 % was considered to be of statistical significance.

### **Phytochemical analysis**

The extract was dark greenish in color with slight characteristic odour, the yield of extract after freeze-drying is 16 g. Phytochemical tests reveal the presence of saponins, phenolics and tannins<sup>10</sup>.

### **Delayed type hypersensitivity response**

The results of DTH response are given in the Table 1. The results indicate that the treatment of *Morus alba* dose dependently increased the DTH response in comparison to control rats. The effect of 400 mg/kg dose of *Morus alba* was found to be comparable with that of the standard drug levamisole.

### Humoral antibody titre

The antibody level determined by hemagglutination technique shows that on the tenth day, for both the dose of *Morus alba*, there is no significant increase in antibody titre as seen from Table 1. The standard drug; however, produces a significant increase in the antibody titre.

### Total leukocyte count

The rats treated with higher dose of aqueous extract of *Morus alba* (400 mg/kg) and the standard drug levamisole (50 mg/kg) produce a significant increase in total leukocyte count as evident from Table 1. A lower dose of aqueous extract of *Morus alba* (200 mg/kg) does not contribute to an increase in the total leukocyte count.

### Differential leukocyte count

The results of DLC from Table 1 reveal that the animals treated with *Morus alba* (200 mg/kg & 400 mg/kg) and the standard drug levamisole (50 mg/kg) generate a considerable increase in the mean percentage of lymphocytes and neutrophils count. *Morus alba* (400 mg/kg) significantly increases the mean percentage of eosinophil count.

**Table 1: Effect of test extracts and standard drug on delayed type hypersensitivity response, humoral antibody titre, total leukocyte count and differential leukocyte count**

| Group                                    | DTH response<br>Mean %<br>increase in<br>paw edema | Humoral<br>antibody<br>titre | TLC<br>count                     | Mean % of<br>lymphocyte | Mean % of<br>eosinophils | Mean % of<br>neutrophils |
|--|--|------------------------------|----------------------------------|-------------------------|--------------------------|--------------------------|
| Control<br>(n = 6)                       | 37.37 ±<br>4.07                                    | 8.00 ±<br>1.79               | 4.88×10 <sup>3</sup><br>± 0.25   | 20.16 ±<br>0.54         | 2.66 ±<br>0.61           | 52.00 ±<br>0.57          |
| <i>Morus alba</i> - 200<br>mg/kg (n = 6) | 47.61 ±<br>1.43*                                   | 14.00 ±<br>4.09              | 5.70×10 <sup>3</sup><br>± 0.26   | 22.50 ±<br>0.34*        | 4.83 ±<br>0.30           | 55.83 ±<br>0.47*         |
| <i>Morus alba</i> - 400<br>mg/kg (n = 6) | 59.70 ±<br>1.29**                                  | 20.00 ±<br>4.00              | 8.4× 10 <sup>3</sup><br>± 0.29** | 25.00 ±<br>0.81*        | 6.16 ±<br>0.40*          | 62.50 ±<br>0.43*         |
| Levamisole - 50<br>mg/kg (n = 6)         | 62.00 ±<br>1.31**                                  | 448.00 ±<br>131.16*          | 9.41×10 <sup>3</sup><br>± 0.67** | 29.00 ±<br>0.81*        | 2.00 ±<br>0.63           | 65.30 ±<br>0.42*         |

\*, \*\* and \*\*\*, denote statistical significance at p < 0.05, p < 0.01 and p < 0.001 vs control

## RESULTS AND DISCUSSION

Aqueous extract of *Morus alba* serves to increase the cell-mediated response but does not produce any change in the antibody titre value. It does not stimulate antibody production and involve primary cell immune response. The earlier immunomodulatory reports on polysaccharide isolated from *Morus alba* root bark in murine splenic lymphocytes have indicated an increased lymphocyte proliferation and decreased antibody production from B cells, which supports our findings<sup>6</sup>.

The DTH response, which measures the cell-mediated immunity (innate or non specific immune response) for the rats treated with 200 mg/kg and 400 mg/kg of *Morus alba* is significant. The mechanism behind the increased DTH could be due to sensitized lymphocytes. Lymphocytes are converted to lymphoblast and secrete variety of molecules, which are known to induce local inflammation with increased vascular permeability, edema and infiltration of PMN leukocytes. The results reveal altered paw edema volume, which substantiate that *Morus alba* mediate DTH response.

There is no change in the antibody level for the animals treated with *Morus alba*. However, there is a significant increase in the antibody titre value with the standard drug. This indicates that *Morus alba* may not have any stimulatory effect on the B cells. B lymphocyte stimulation is required for agglutination (Specific or Adaptive immunity). Further, it is found that on stimulation by antigen, there is a significant increase in total leukocyte counts and differential leukocyte counts. Possibilities are there that the increased number of leukocytes, eosinophils, lymphocytes and neutrophils could have been responsible for the increased DTH response in rats.

It is thus concluded that *Morus alba* aqueous extract stimulates the innate or non specific immune system in a dose dependant manner and does not stimulate the adaptive immune system in mediating immunomodulatory property.

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