



## Immunosuppressive activity of aqueous extract of *Lagenaria siceraria* (standley) in mice

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### Abstract

The immunosuppressive activity of the Aqueous extract of fruit of *Lagenaria siceraria* consisting of mixture of saponins, flavonoids, tannins, steroids, phenol and glycosides was studied in mice. The assessment of immunomodulatory activity was carried out by testing the humoral (antibody titre) and cellular (foot pad swelling) immune responses to the antigenic challenge by sheep RBCs. Oral administration of extract showed a significant decrease delayed type hypersensitivity response whereas the humoral response to sheep RBCs was unaffected. Thus the extract significantly suppressed the cellular immunity by decreasing the footpad thickness response to sheep RBCs in sensitized mice. With a dose of 150 and 300 mg/kg/day the DTH response was  $7.66 \pm 2.75$  and  $6.47 \pm 1.12$  respectively in comparison to control group  $14.25 \pm 2.48$  ( $P < 0.05$ ). The study demonstrates that the extract shows preferential suppression of the components of cell-mediated immunity and shows no effect on the humoral immunity.

**Keywords:** Immunosuppressants, *Lagenaria siceraria*, Delayed type hypersensitivity, Hemagglutinating antibody titer, aqueous extract.

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### Introduction

Clinical transplant immunosuppression aims not only to prevent host immune responses against antigens on the transplanted organ, thereby avoiding rejection, but to prevent undesirable complications of immunodeficiency (eg, infection and malignancy) and to minimize nonimmune toxicities (eg, nephrotoxicity, hyperlipidemia, bone marrow suppression, and cushingoid effects). While physical methods such as irradiation can be used, in practice, immunosuppression for solid organ transplantation is usually achieved by immunosuppressive drugs (ISDs). It is an interesting paradox that many of the currently used ISDs, while responsible for drastic improvement in short-term outcomes, potentially compromise long-term graft and patient survival through complex toxic mechanisms.

Therefore, modification of immune and nonimmune responses to ISDs through individualization of immunosuppressive agents and regimens for specific patients and groups of patients is a major priority. Clinicians caring for transplant recipients must consider the evidence for the best outcomes with the lowest toxicity. Some of the plants employed in traditional medicine were shown to possess immunosuppressive activity, *Cordyceps sinensis* [1] and *Allium cepa*[2] are two examples of such plants.

The plant, *Lagenaria siceraria* (family: Cucurbitaceae), known as bottle gourd, is a common fruit vegetable used throughout India. Since time immemorial the fruit is used as immunosuppressant, diuretic, cardio-tonic, cardio-protective and nutritive agent. The fruit is also reported to have good source of vitamin B complex and choline along with fair source of vitamin C and  $\beta$ -carotene [3]. It is also reported to contain Cucurbitacins, fibers and polyphenols [4]. Two sterols namely Campesterol and Sitosterol have been identified and isolated from the petroleum ether fraction of methanol extract of *Lagenaria siceraria* fruits, which is reported to possess antihepatotoxic activity [5]. The fruit has been reported to possess antioxidant activity [6], hypolipidemic and triton-induced hyperlipidemic rats [7]. HPLC analysis of methanolic extract from plant shows the presence of flavones-c glycosides [8]. Lagenin, a novel protein has been isolated from lyophilized extract of seeds [9]. The present hypothesis tested the immunosuppressive activity of aqueous extract of fruit of *Lagenaria siceraria* to prove its traditional medicinal importance.

## Materials and Methods

### ***Preparation of aqueous extract of Lagenaria siceraria (AELS)***

The fruit of *Lagenaria siceraria* was collected in the month of Dec 2008 from Tambaram, Chennai. Identification and Authentication of fruit was done by Prof. Jairaman, Ph.D (Botanist) Tambaram (PARC-2009-217). The fresh and semi-ripened fruits were cut into small pieces and fed to a juicer to collect the juice and the collected juice was filtered and vacuum dried to obtain the *L. Siceraria* fruit juice extract and the yield was about 17% w/w.

### ***Phytochemical screening***

The presence of phytochemicals alkaloids (Dragendorff's), flavonoids (Shibata's reaction), saponins (Frothing test), tannins (10% ferric chloride), terpenoids (2,4-dinitrophenylhydrazine), glycosides (Fehling's solution), steroids (Liebermann's Burchard test) were evaluated.

### ***Animals***

Healthy male albino mice (25-30g) were selected for the study. Animals were housed in standard isolation cages (45×35×25 cm) under environmentally controlled conditions with 12-h light/12-h dark cycle. Mice were allowed free access to water, standard laboratory rat chow (Hindustan Liver Pvt. Ltd, Mumbai) throughout the experiment. Fresh sheep red blood cells (SRBC) in Alsever's solution were prepared in host department after collecting fresh sheep blood from local slaughter house.

### ***Antigen***

SRBC collected in Alsever's solution, were washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of  $0.5 \times 10^9$  cells/ml for immunization and challenge.

### ***Treatment***

The animals were divided into four groups consisting of six animals each. A group of six

untreated mice were taken as control (Group I). The aqueous extract of *Lagenaria siceraria* (AELS) was fed orally for 14 days at a dose of 50 mg/kg/ day (Group II), 150 mg/kg/day (Group III) and 300 mg/ kg/day (Group IV) for assessment of immunomodulation effect. The animal experimental protocols were approved by the Institute Animal Ethics Committee.

#### ***Haemagglutinating antibody (HA) titer***

Haemagglutinating antibody titre was determined according to the method of Puri *et al* [10]. Mice of group II, III and IV were pretreated with AELS for 14 days and each mouse was immunized with  $0.5 \times 10^9$  SRBC/mouse by *i.p.* route, including control mice. The day of immunization was referred to as day 0. The animals were treated with AELS for 14 more days and blood samples were collected from each mouse on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBC ( $0.025 \times 10^9$  cells). The microtitre plates were incubated at room temperature for two hours and examined visually for agglutination. The highest number dilution of serum showing haemagglutination has been expressed as HA titre.

On 15<sup>th</sup> day of treatment, all the mice were sacrificed and blood was collected in heparinized vials. Blood samples for animals of each group were subjected for hematological studies such as total WBC count and spleen leukocyte count. Spleen and thymus were dissected out and embedded in 10% formalin solution to record their weight.

#### ***Delayed type hypersensitivity (DTH) response***

Six animals per group (control and treated) were immunized on day 0 by *i.p.* administration of  $0.5 \times 10^9$  SRBC/mouse and challenged by a intraplantar administration of  $0.025 \times 10^9$  SRBC/ml into right hind foot pad on 7<sup>th</sup> day. The AELS was administered orally from day 1 until day 7. DTH response was measured at 24 h after SRBC challenge on day 8 and expressed as mean percent decrease in paw volume (Plethysmometrically) [10].

#### ***Statistical analysis***

The data were analysed using One-way analysis of variance (ANOVA) followed by Dunnett test. P values <0.05 were considered significant.

### **Results**

The phytochemical screening of the AELS revealed the presence of phytochemical constituents such as saponins, flavonoids, tannins, steroids, phenol and glycosides.

The results of HA titre and DTH response are shown in Table 1. Even with the administration of increasing doses of AELS, the HA titre did not show any significant increase as compared to untreated control group indicating that the *Lagenaria siceraria* had no effect on humoral immunity.

The DTH response to SRBC which corresponds to cell mediated immunity showed a significant dose dependent decrease due to treatment with AELS with dose of 150 mg/kg/day and 300 mg/kg/day. The DTH response was  $7.66 \pm 2.75$  and  $6.41 \pm 1.12$  respectively in comparison to corresponding value of  $14.50 \pm 2.38$  for untreated control group. The dose dependant differences in DTH response were statistically significant ( $P < 0.05$ ).

**Table 1. Effect of *Lagenaria siceraria* on HA titer and DTH response to antigenic challenge by sheep RBCs in mice**

Groups (% decrease in paw volume)	HA titre	DTH response
I (Untreated)	5.20±0.22	14.50±2.38
II (50 mg/kg, <i>p.o.</i> )	5.09±0.81	10.52±3.12*
III (150 mg/kg, <i>p.o.</i> )	5.16±0.75	7.66±2.75**
IV (300 mg/kg, <i>p.o.</i> )	4.90±0.63	6.41±1.21**

The values are mean±SD of 6 mice in each group. One-way ANOVA followed by Dunnett's multiple comparisons test; \*P<0.05, \*\*P<0.01 Vs group I.

Thus AELS treatment induced marked inhibition of DTH response to SRBC in the animals. Finally, the effects of AELS on WBC, spleen leukocytes count and relative organ weight in mice are shown in Table 2. AELS at the dose of 150 mg/kg and 300 mg/kg., *p.o* caused a significant reduction in the WBC, Spleen leukocyte counts. But the effect was more pronounced at dose of 300 mg/kg (P<0.01) as compared to 150 mg/kg *p.o* dose of AELS (P<0.05).

**Table 2. Effect of *Lagenaria siceraria* on WBC, spleen leukocytes count and relative organ weight in mice**

Groups	WBC (thousand/cmm)	Spleen leukocyte (thousand/cmm)	Thymus weight (g/100 g B.W)	Spleen weight (g/100 g B.W)
I (Untreated)	11.3±0.47	48.5±9.9	0.08±0.02	0.35±0.041
II (50 mg/kg, <i>p.o.</i> )	10.9±0.38 <sup>ns</sup>	42.9±8.4 <sup>ns</sup>	0.07±0.02 <sup>ns</sup>	0.31±0.027 <sup>ns</sup>
III (150 mg/kg, <i>p.o.</i> )	10.6±0.45*	35.1±7.2*	0.05±0.02*	0.29±0.031*
IV (300 mg/kg, <i>p.o.</i> )	9.54±0.46**	28.4±6.5**	0.03±0.01**	0.23±0.027*

The values are mean±SD of 6 mice in each group. One-way ANOVA followed by Dunnett's multiple comparisons test; <sup>ns</sup>P>0.05, \*P<0.05, \*\*P<0.01 Vs group I.

## Discussion

A wide range of immunosuppressive drugs have now been adopted to control unwanted immune responses, particularly those giving autoimmune disease and transplant rejection. The clinical application of immunosuppressants has significantly improved patient survival with first-year survival up to 90% for renal transplant [11]. But unfortunately immunosuppressants are suffers from a number of serious adverse effects among which

nephrotoxicity, hepatotoxicity, induction of diabetes, induction of hypertension and neurotoxicity are most notorious for cyclosporine and tacrolimus [12]. As a consequence, there continues to be a high demand for new immunosuppressants. The immunosuppressants without any side effects are still a challenge to the medical system. Suppression of immune response by medicinal plant products as a possible therapeutic measure has become a subject of scientific investigation recently [13]. In an effort to search for new immunosuppressants, we identified clinically useful and safe product from medicinal plants that could suppress immune response and may have future in clinic. This study reported the effect of AELS on the humoral and cellular immune responses to mice subcutaneously immunized with SRBCs. In the experiments undertaken to study the effect of AELS on haemagglutination antibody titre against SRBC, it was observed that even with the administration of increasing doses of AELS, the titre did not show any significant increase as compared to untreated control group indicating that the AELS had no effect on the humoral immunity.

The DTH response, which is a direct correlate of cell mediated immunity (CMI), was found to be significantly decreased at a dose of 150 and 300 mg/kg/day of the AELS. During CMI responses, sensitized T-lymphocytes, when challenged by the antigen, are converted to lymphoblast and secrete lymphokines, attracting more scavenger cells to the site of reaction. The infiltrating cells are thus immobilized to promote defensive (inflammatory) reaction. In our studies, foot volume was decreased after AELS treatment suggesting cell mediated immune suppression [14]. In the DTH response, AELS had inhibitory effect on lymphocytes and accessory cell types required for the expression of the reaction [15]. This supports the reported anti-inflammatory activity of AELS [16]. Here it is interesting to note that the treatment, while augmenting the CMI, did not affect the antibody titres.

## Conclusion

In the present study, the immunosuppressant activity of *Lagenaria siceraria*, an important plant in indigenous medicinal practice was explored. Administration of *Lagenaria siceraria* was found to decrease total WBC count and spleen leukocyte count significantly indicating that the extract could suppress the non-specific immune system.

## Acknowledgement

Authors are grateful to Dr. K.S Lakshmi, Dean & Dr. K. Ilango, Vice Principal, College of Pharmacy & SRM University, Kattankulathur, for providing necessary facilities to carry out this work.

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