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Phytochemical investigation and immunomodulatory activity of *Lagenaria siceraria* fruits

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Abstract

An attempt has been made to assess the immunomodulatory activity of methanolic extract of *Lagenaria siceraria* fruits at three dose levels ranging from 100-500 mg /kg body weight in rats using haemagglutination antibody titre, *in vivo* phagocytosis and cyclophosphamide-induced myelosuppression. Methanolic extract increased haemagglutination antibody titre and carbon clearance significantly. It also prevented myelosuppression in rats treated with immunosuppressive drug-cyclophosphamide. A significant increase in white blood cell count was observed in methanolic extract - treated rats as compared with cyclophosphamide treatment alone. Methanolic extract and its different fractions were also tested phytochemically. It is concluded that *Lagenaria siceraria* fruits possess immunomodulatory activity.

Key words: Myelosuppression, Immunomodulation, *Lagenaria siceraria*, Phytochemical Screening.

1. Introduction

Indian medicinal plants are rich source of substances that are claimed to induce immunity [1]. *Lagenaria siceraria* (Molina) Standley (family cucurbitaceae) commonly known as lauki (Hindi) and bottle gourd (English) is a medicinal plant [2] and fruits of this plant are traditionally used for their cardioprotective, cardiotonic, general tonic, aphrodisiac, diuretic and nutritive properties [3]. Fruits are also used in treatment of pain, ulcer, fever, pectoral cough, asthma and other bronchial disorders [4]. However there is paucity of data available on the effect of the extract of *Lagenaria siceraria* fruits on humoral immune response, cyclophosphamide - induced myelosuppression and phagocytic function of the cells of the reticuloendothelial system. Therefore, the present study was undertaken to investigate the immunomodulatory effect of methanolic extract of *Lagenaria siceraria* fruits.

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2. Materials and methods

2.1. Plant material

The fresh fruits of *Lagenaria siceraria* were collected from outfield of Junagadh city, Gujarat state, India in March 2007. Plant was authenticated by the authority of department of botany, Bahauddin college, Junagadh, where a voucher specimen (BS/Bot./I-5/06-07) has been deposited for future reference.

2.2. Preparation of extract

The coarsely powdered plant material (500g) was defatted with petroleum ether and then extracted with methanol in a Soxhlet extractor. The methanol extract was concentrated by distilling off the solvents and evaporated to dryness using water bath. The residue (69g) was then enriched in flavonoids and saponins by suspending in water and extracting successively with ethyl acetate and n butanol (3×300 ml each). Resulting solutions were then concentrated to provide ethyl acetate soluble (3.2g), n butanol soluble (14.5g) and water soluble (47.3g) portions.

2.3. Phytochemical screening

Methanolic extract, its ethyl acetate and n butanol soluble fractions were tested for the presence of alkaloids, saponins, tannins, flavonoids, anthraquinones and sterols according to standard procedures [5].

2.4. TLC fingerprint profile

TLC (silica gel G 60 F_{254} TLC plates of E. Merck, layer thickness 0.2mm) fingerprint profile was established for the ethyl acetate and n butanol soluble fractions of methanolic extract of *Lagenaria siceraria* fruits. Samples were spotted on TLC plates using Camag Linomat V automatic sample spotter. Solvent systems; n butanol: acetic acid: water (4:1:5) and chloroform: methanol (90:10) were used respectively for ethyl acetate and n butanol fractions. The plates were scanned using TLC scanner 3 (Camag) at 254nm (absorption mode) & 366nm (fluorescence mode) for n butanol fraction and 290nm (absorption) & 600nm (absorption mode) for ethyl acetate fraction. Rf values and % relative areas were recorded from the scanned area. Developed chromatograms were then sprayed with vanillin sulphuric acid and heated at 100°C for 10 min.(n butanol fraction) and with 1% aluminium chloride solution in methanol and observed under uv (ethyl acetate fraction).

2.5. Experimental animals

Wister albino rats of either sex and weighing 150 to 180 g were housed in groups of four per cage under controlled light (12:12 light: dark cycle) and temperature (25 ± 20C) environment and behavioral assessment was conducted during the light cycle. Food (Pranav agro sales, Ahmedabad) and water was provided *ad libitum*. All procedures were carried out under strict compliance with ethical principles and guidelines of the Institutional Animal Ethical Committee constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experimental Animals, Madras. All the animals survived the toxicity studies at all dose levels. Based on the study; doses of 100, 200 and 500 mg/kg were selected for animal experiments.

Methanolic extract of *Lagenaria siceraria* fruits was suspended in 1% sodium carboxy methyl cellulose (SCMC) to prepare different doses from 100 to 500 mg/kg. The control animals were given an equivalent volume of SCMC vehicle. Cyclophosphamide was used as a standard immunosuppressant. Carbon ink suspension: Pelican, Germany, ink was diluted eight times with saline and used for carbon clearance test (*in vivo* phagocytosis).

2.6. Antigen

Fresh sheep blood was collected from local slaughter house in Alsever's solution. During the experiment, adequate amount of stock solution

Table 1. Effect of methanolic extract of *Lagenaria siceraria* fruits on SRBC-induced haemagglutination antibody titre in rats

Group	Treatment (mg/kg)	Mean haemagglutination antibody(HA) titre Range	1^0 HA titre	Range	2^0 HA titre
Control (sensitized)	-	0 - 32	32 ± 0.00	32 - 64	37.33 ± 5.33
ME	100	64 - 128	117.33 ± 10.66[a]	64 - 128	117.33±10.66[b]
ME	200	128 - 256	149.33 ± 21.33[a]	64 - 128	106.66 ± 13.49[c]
ME	500	128 - 512	234.66 ± 61.08[b]	128 - 256	213.33 ± 26.98[b]

Values are mean ± SEM, a p>0.05, b p<0.01, c p< 0.05, compared to control group animals, (n=6), 10 primary, 20 secondary, ME methanolic extract.

Table 2. Effect of methanolic extract of *Lagenaria siceraria* fruits on *in vivo* phagocytosis and on cyclophosphamide–induced myelosuppression in rats

Group	Treatment (mg/kg)	Phagocytic index (K)	Total WBC (10^3/mm^3) Day 0	Total WBC (10^3/mm^3) Day 14
Control	-	0.0221 ± 0.000	9.99 ± 0.16	10.4 ± 0.51
ME+CP*	100	0.0228 ± 0.001[a]	10.15 ± 0.23	6.99 ± 0.34[a]
ME +CP*	200	0.0326 ± 0.001[b]	10.11 ± 0.12	8.85 ± 0.38[c]
ME +CP*	500	0.0438 ± 0.001[b]	10.15 ± 0.21	10.78 ± 0.28[b]
CP	30	-	9.97 ± 0.19	6.32 ± 1.09

Values are mean ± SEM, [a] p>0.05, [b] p< 0.01, [c] p< 0.05 compared to control group animals or cyclophosphamide treatment alone, (n=6), CP cyclophosphamide.* CP with ME administered to only those rats undergoing cyclophosphamide–induced myelosuppression study; remaining animals only administered with ME.

(Sheep red blood cells, SRBCs, stored in Alsever's solution) was taken and allowed to stand at room temperature. It was washed three times with normal saline. The settled SRBC was then suspended in normal saline and RBC of this suspension was adjusted to a concentration of 5x109 /ml for immunization and challenge [6].

2.7. SRBC - induced humoral antibody (HA) titre [7]

Groups of six rats per treatment were immunized by injecting 20 µl of SRBC suspension (5x109 SRBC /ml) subcutaneously into right hind foot pad. Seven days latter they were challenged by injecting 20 µl of SRBC suspension (5x109SRBC /ml) intradermally into the left hind foot pad. The day of immunization was referred to as day 0. Blood samples were collected from all the animals separately by retro orbital puncture on day +7 (before challenge) for primary antibody titre and on day +14 for secondary antibody tire. Antibody levels were determined by the method described by Shinde *et al.* [8]. Briefly 25 µl aliquot of serum of each animal was taken in microtitre plates. To serial two - fold dilutions of pooled serum (made in 25 µl normal saline), 25 µl of 1% v/v SRBC suspension (in normal saline) was added. The microtitre plates were kept at room temperature for 1 h and then observed for haemagglutination (until control wells showed unequivocally negative pattern). The value of the highest serum dilution showing haemagglutination was taken as the antibody titre. The methanolic extract was

fed orally once daily, starting with 7 days prior to sensitization till the challenge (-7,-6,-5,-4,-3,-2,-1,0,+1,+2,+3,+4,+5,+6,+7).

2.8. In vivo phagocytosis [9]

Animals of the treatment group were given methanolic extract daily for 5 days. At the end of five days, after 48 hrs., rats were injected via tail vein with carbon ink suspension (10 µl/ gm body weight).Blood samples were drawn (in EDTA solution, 5 µl) from the retro orbital vein at 0 and 15 min.; a 25 µl sample was mixed with 0.1%sodium carbonate solution (2 ml) and its optical density was measured at 680 nm. The phagocytic index (K) was calculated using the equation: K= (logOD1-logOD2)/ 15 Where OD1 and OD2 are optical densities at 0 and 15 min.

2.9. Cyclophosphamide-induced myelosuppression [10]

Animals were divided into five groups of six animals each. Group I (control group) and group V (cyclophosphamide treated control) received the vehicle for a period of 13 days. Groups II-IV were given methanolic extract daily for 13 days. The animals of group II-V were injected with cyclophosphamide (30 mg/kg, i.p.) on the 11th, 12th and 13th day, 1 h after the administration of the respective treatment. Blood samples were collected on the day 0 and on the 14th day and total white blood cell (WBC) count was determined by using a haemocytometer.

2.10. Statistical analysis

The values are expressed in mean ± SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's *t* test to determine the statistical significance. $P < 0.05$ was accepted as statistically significant.

3. Results

Preliminary phytochemical screening of methanolic extract showed the presence of flavonoids, saponins, sterols, tannins and carbohydrates. n butanol soluble fraction found to contain sterols and saponins while flavonoids were found in ethyl acetate soluble fraction of methanolic extract. Methanolic extract of *Lagenaria siceraria* fruits significantly increased haemagglutination antibody titre. Primary antibody titre was found to be increased in a dose dependent manner [Table1]. The extract also increased rate of carbon clearance from rats significantly, indicating increased *in vivo* phagocytosis [Table 2].

Methanolic extract prevented myelosupprrssion in rats treated with immunosuppressive drug-cyclophosphamide. A significant increase in white blood cell count was observed in methanolic extract treated rats as compared to untreated (control) rats [Table 2].

4. Discussion

The results obtained in the present study showed that the extract displays a dose dependent immunomodulatory effects. Antibody molecules, a product of B lymphocytes and plasma cells, are central to humoral immune response; IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxin etc. The antibody production of T dependent antigen, SRBCs, requires the co-operation of T and B-lymphocytes and macrophages. The augmentation of humoral response by methanolic extract, as evidenced by an enhancement of antibody responsiveness to SRBC in rats as a consequence of both pre and post immunization extract treatment, indicates the enhanced responsiveness of macrophages and B lymphocytes subsets involved in antibody synthesis [11]. A high degree of cell proliferation renders the bone marrow a sensitive target particularly to cytotoxic drugs. In fact, bone is the organ most affected during any immunosuppression therapy with this class of drugs. Loss of stem cells and inability of the bone marrow to regenerate new blood cells

results in thrombocytopenia and leucopenia. Administration of methanolic extract of *Lagenaria siceraria* fruits increased the total WBC count, which was lowered by cyclophosphamide. The results of the present study indicate that the test drug can stimulate the bone marrow activity [10].

Phagocytosis by macrophages is important against microorganism and its effectiveness is markedly enhanced by opsonization of parasite with antibody and complement C3b leading to more rapid clearance of parasite from blood[6]. Administration of methanolic extract enhanced the carbon clearance rate from circulation in rats significantly compared to animal of control groups. High performance thin layer chromatography was performed to further separate components from different fractions of methanolic extract of *Lagenaria siceraria* fruits. TLC fingerprint profile was established for the varied fractions of bioactive methanolic extract. Phytochemical screening has shown the presence of flavonoids, saponins and carbohydrates. Saponins and flavonoids have long been recognized to possess a wide variety of biological activities including immunomodulatory property, so the primarily role of these secondary metabolites in the said pharmacological activity can not be ruled out [12-15]. The present study with *Lagenaria siceraria* fruits leads us to conclude that methanolic extract augments humoral immune response, activates macrophage-induced phagocytosis and prevents cyclophosphamide-induced myelosu-ppression.

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