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Immunomodulatory Potential of Gemmomodified Extract of *Terminalia arjuna*

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Abstract:*.In recent years there is increasing interest in the field ofmodulation of immune by plant products. The aim of this study was to evaluate the immunomodulatory potential of bark and gemmomodified extracts of Terminalia arjuna.Gemmotherapy is a new form of therapy in which super active form of medicines, made from freshly growing parts are used for the treatment of various diseases. Effect of extracts of T. arjuna on cell-mediated and humoral immune reposes was evaluated by phagocytosis and haemagglutination assays respectively.Macrophages engulfment and antibody production was stimulated after the induction of both extract of T. arjuna however, bark extract showed slightly greater immunostimulatory effect as compared to gemmomodified extract. Both extracts of Terminalia arjuna (methanolic bark and gemmo modified) were found considerably effective in boosting cell mediated (Phagocytosis) and humoral immune response.*

Key Words:*Phagocytosis, Terminalia arjuna, Immunomodulatory*

I. Introduction

The immune system is defense system of the body that provides protection against foreign invaders, which are disease causing microbes (bacteria, parasite and fungi). Plant based drugs have been intensified all over the world. One of the main strategies of herbal treatment is to increase the body's natural resistance to disease/stress causing agents rather than directly neutralizing the agents itself in practice. Plant origin immunomodulatory agents enhanced para immunity, non specific immunomodulation of granulocytes, macrophages, natural killer cells and complement functions[1-6].

Immune diseases are big problem for mankind. Immunomodulatory therapy is recognized as important alternate to conventional chemotherapy in many diseases. Use of plant compounds to enhance the phagocytosis ability of macrophages and increase antibody production has been well documented by many researchers[2,4,5,7-9]. The use of plant products as immunomodulator is still in developing stage[10-12].A variety of phytochemicals such as polysaccharides[13]and flavonoids[14] have been reported to modulate the immune system.

Beside conventional herbal medicines gemmotherapy is emerging and less studied field. Gemmotherapy is fabulous active form of medicine, made from embryonic tissues of various trees and shrubs (buds and young shoots), reproductive parts and newly grown tissues. The powerful medicinal potential of fresh germinating parts of plants is getting attention of scientists working in various disciplines[15]. *Terminalia arjuna* is versatile traditional medicinal plant Clinical studies suggested that *T. arjuna* is effective in patient of stable angina[16]. It improves the blood circulation to heart, regulate blood pressure[17]used for treatment of hypercholesterolemia[15,18-19] and inhibited the platelet aggregation[20].

Little work has been done on immunomodulatory activity of *Terminalia arjuna*.It is need of time to develop alternative therapies for treatment of immune disease therefore, this project has been designed to explore the important immunomodulatory potential of gemmomodified and bark extracts of *Terminalia arjuna.*

II. Materials and methods

Extract preparation

Bark extract

The bark powdered (30 g) of *Terminalia arjuna*was refluxed with methanol. After completion of time extract was filtered and methanol was evaporated under reduced pressure to get crude extract and stored in refrigerator at 4°C for further use in analysis

Gemmo modified extract

Paste of plant material (100g), which is freshly harvested from plants during their growing stage was macerated with one liter mixture of glycerin and methanol in a ratio of 1:2 and shake vigorously. After one month macerate was filtered and solvent was removed with rotary evaporator and crude extract was stored.

Evaluation of Phagocytosis Activity

Different steps involved in evaluation of cell phagocytosis assay are as follow:

Collection of Abdominal Exudate Cells

The abdominal exudate cells (AEC) were acquired from the grown-up (age 4 weeks) chickens. Sephadex granules G-50 (3%, w/v) were suspended in normal saline solution (0.85%). Suspension of Sephadex granules was injected into chickens intra peritoneally at the dose of one mL per 100 g b.wt. After 48 hrs of Sephadex injection, the abdominal cavity was lavaged with heparin (0.5 U/mL) and normal saline (0.85%). AEC were obtained aseptically from three different chickens and combined for further investigation. AEC suspension was washed two times with medium (M-199, Merck USA), the medium was accessorized with fetal calf serum (5%) and two antibiotics including penicillin (100 unit /mL) and streptomycin (50 µg /mL). The viability of AEC was confirmed with the use of trypan blue (dye) and the concentration of $1x10^6$ AEC per mL was finally preserved in sterilized RPMI-1640 medium[21] (SANDHU et al., 2006).

Preparation of Extract Dilutions

Three different concentrations of *Terminalia arjuna*extracts from were diluted serially as 10 fold dilutions. Sterilized double distilled water was used for the preparation of dilutions. Double distilled water (9 mL) was taken in three test tubes separately. One mg of respected extracts was transferred into 1st test tube and mixed vigorously at ambient temperature. One mL of the content from first test tube was transferred to second test tube, mixed for one min at ambient temperature and one mL solution was again transferred from 2nd tube into the 3rd test tube maintaining 10 fold serial dilutions and each dilution contained 100, 10, 1 µg/m L. of plant extract respectively. All the diluted extract samples were stored below 10°C for further use.

Phagocytosis Assay

Phagocytosis assay was performed according to the method reported by SANDHUetal. (2006). For this assay sterilized petri dishes with five round glass cover slips per extract was prepared in duplicate. Already filtered and sterilized RPMI-1640 cell culture medium (15 mL) was transferred into Pyrex glass petri dishes. Two mL of AEC suspension was shifted to each petri dish slightly mixed and kept under incubation with 10% CO_2 into incubator for 3 h.

Glass cover slips were washed with sterilized RPM1-1640 medium and maintained into freshly changed medium in the Petri dishes. One Petri dish for each group was reserved as untreated controlled where as the other petri dishes having five glass cover slips was provided with one mL of diluted phenolics fractions, after gentle mixing, the petri dished were kept for incubation at 37°C for 45 minutes. Washed sheep RBCs suspension (2 mL, 1 %) was transferred separately in all Petri dishes and further incubated for 1 h at 37°C. The cover slips were separated from culture medium and unphagocytos RBCs was removed by single washing with normal saline (0.85 %). The macrophages cells were preserved in methanol and the stained with the help of Dip Quick and placed on clean glass slides Engulfed macrophages were calculated according to formula:

$$\% \text{ of macrophages engulfed} = \frac{\text{Average number of RBCs engulfed by macrophages}}{\text{Average no. of adhering macrophages}}$$

Determination of Humoral Antibody Titer

The effect of *Terminalia arjuna* extractson antibody response was evaluated by the humoral antibody response in rabbits against sheep RBCs that served as non specific antigen in treated and untreated experimental groups.

Experimental Protocol

Male albino rabbits were selected for this study and animals were kept under standard condition of environment, water and diet. Rabbits were divided into different experimental groups

Group 1; normal controls; rabbits were administered standard diet only.

Group 2; .Antigen control, rabbits of this group was immunized with SRBC (sheep red blood cell) injected subcutaneously on 1st and 3rd day.

Group 3; 100 mg/kg plant extract was given to this group daily for 14 days

Group 4; 200mg/kgbw plant extract was given daily for 14 days.

Group 5; these rabbits were first immunized with RBCs on 1st and 3rd day after immunization the plant extract (100 mg/kg b.wt)was given for 14 days.

Group 6; Rabbits were treated as group 5 then dose of plant extract (200mg/kgb.wt) was given for 14 days

Haemagglutination Assay

Serum was separated from blood of different experiment groups at the end of experimental period. Antibody titer was evaluated by Haemagglutination assay. Serial bifold dilution of serum in 96 wall micro titer plates was

prepared with normal saline, then fresh SRBCs (1%, 25 ul) was shifted into each dilution and mixed softly then left the plates for 1 h. After completion the time anti body titer in terms of highest dilution of serum sample with haemagglutination was noted. Mean $\log_2$ of the titer was determined to compare the results[10].

Statistical Analysis

Each sample was analyzed in triplicate and data is expressed as mean ± SD.

Data was analyzed using analysis of variance ANOVA in SPSS 15 software. Tukey Multiple Comparison test was used for comparison of means of different experiments ($p < 0.05$)

III. Results & Discussion

Effect of Plant Treatment on Phagocytosis

Peritoneal macrophages treated with different doses of plant extracts were evaluated for their capabilities to digest and ingest particle and have been shown in (Fig 1). The treatment with methanolic bark and gemmo modified extract of *Terminalia arjuna* significantly increased the number of SRBC engulfed by macrophages in dose dependent manner. Macrophages engulfed 25 ± 2.91 and 23.2± 3.115 SRBC after induction of bark and gemmomodified extract of *Terminalia arjuna* respectively at the concentration of 100 µg/mL. Number of engulfed cells was significantly higher ($p < 0.05$) than untreated control (1.6 ± 0.25). Moreover, after the treatment with both extracts of *Terminalia arjuna* bark and gemmomodified the macrophages engulfment percentage (% phagocytosis) was significantly ($p < 0.05$) higher (95.367 ± 1.9, 93.9 ± 1.00 %) than control (39.8 ± 0.40 % Fig # 2). Significant immune stimulatory effect of *Terminalia arjun* extract

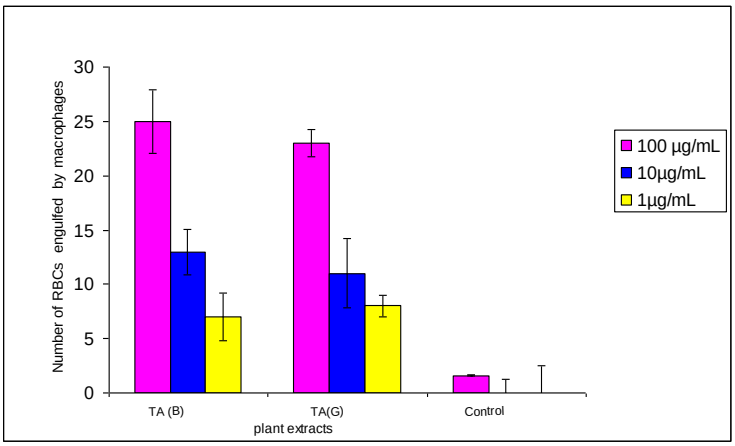

Fig 1. Macrophages engulfment factor with different concentrations of *Terminalia arjuna* extracts

All values expressed as mean ± SD (n=3) Significant. *Terminalia arjuna* Bark extract, TA (G) = *Terminalia arjuna* gemmo extract

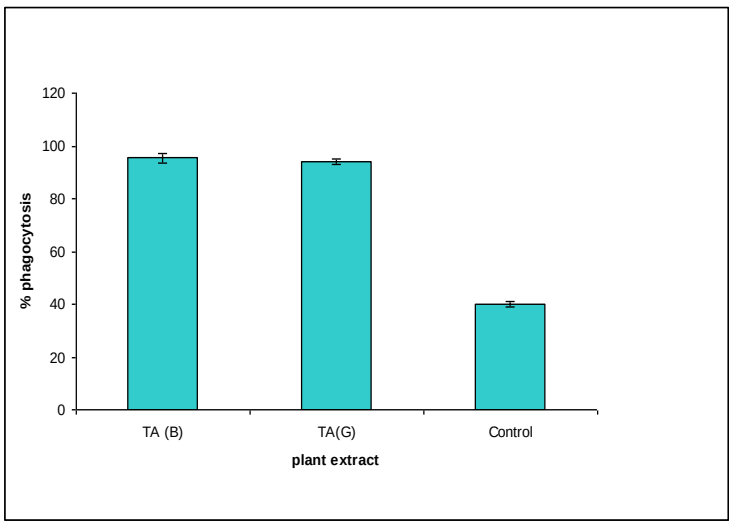

Fig 2. Percentage (%) Phagocytosis after treatment with extracts of *Terminalia arjuna*

All values expressed as mean ±SD (n=3). TA (B)=*Terminalia arjuna* Bark extract, TA (G) = *Terminalia arjuna* gemmo extracton Phagocytosis was observed when compared with control.

Effect of Different Plant Extract on Humoral Antibody Titer

Both extracts exhibited stimulation of antibody production dose dependently, but different in extent (Table 1). *Terminalia arjuna* bark extract (200 mg/kg b.wt) significantly ($p<0.05$) increased the humoral antibody titer (11.5 ± 2.062) after immunization of rabbits with RBCs in comparison of normal control (4.25 ± 0.50) and antigen control (6.5 ± 0.57). Mean antibody titer for gemmo modified extract of *Terminalia arjuna* treated rabbits was 11.00 ± 1.00, which was significantly ($P<0.05$) higher than control (4.25 ± 0.50). *Terminalia arjuna* both gemmomodified and bark extracts showed almost similar effect on antibody titer.

Modulation of immune system (stimulation and suppression) is important strategy in therapy of many diseases. A number of research studies have been shown plant product's multiple actions on defense system responses[4,6,12,22-24,30].Macrophages are involved in the commencement of different immune response including innate and adaptive immunity. They play crucial role to engulf and digest (phagocytose) cellular trash and microbial invaders. Phagocytosis is important ability of macrophages, and when this is disturbed it results in increase risk of infections. Antibodies are specific immune proteins and their production is major function of immune system as they have ability to combine with antigens that can activate their production. The antibody titer is helpful to determine the changes in the quantity of antibodies in the route of immune reaction. Humoral antibody titer as well as cell mediated immunity (Phagocytosis) are very important parameters to check the efficiency of immune system.

Both extracts of *Terminalia arjuna* (methanolic bark and Gemmo modified) were found to be substantially effective in boosting cell mediated (Phagocytosis) and humoral immune response (antibody titer). The treatment of the both extracts of *Terminalia arjuna* methanolic bark and gemmo modified at different level of doses (100, 200 mg/kg b.wt.) increased the antibody production and significantly enhanced the phagocytosis ability of macrophages. Humoral antibody titer and phagocytosis are important parameter to evaluate the effect of medicinal plants on immune system.Many scientists reported the effect of plant extracts on antibody titer and phagocytosis activity[5,25-27]. The increase in engulfment ability of macrophages and antibodies production may be due to the immunostimulatory effect of *Terminalia arjuna* extracts. Immunomodulatory effect of medicinal plants has been attributed to polyphenols (antioxidant components). Flavonoids and polyphenols have been renowned to possessImmunomodulatory potential[28]. Different studies confirmed the strong antioxidant behavior and high polyphenolic contents of*Terminalia arjuna*. Significantly higher immunostimulatory effect of *Terminalia arjuna* links to its higher antioxidant power and polyphenolic contents. These results are in agreements with some previous reports about immunostimulatory effect of polyphenols[9].

Immunomodulatory effect of *Terminalia arjuna* was reported in literature but no scientific study was available in this regard and this study can provide scientific validation of immunostimulatory potential of *Terminalia arjuna.* From these results it is concluded *Terminalia arjuna*exhibited high immunostimulatory effect. Both extracts boosted the cell-mediated as well as humoral immune responses.

Table 1 Effect of *Terminalia arjuna* extracts treatment on humoral antibody Titer in different experimental groups of rabbits

Groups **HA titer**	**G1** **Normal Control**	**G2** **(Positive control)**	**G3** **100 mg/kg (Plant extract)**	**G4** **200 mg/kg (Plant extract)**	**G5** **100 mg/kg (Plant extract + antigen)**	**G6** **200 mg/kg (Plant extract + Antigen)**
T. arjuna *(bark)*	4.250±0.500	6.5±0.577	4.7±50.500	5.0±0.578	10.75±0.577*	11.5±2.062**
T. arjuna(*gemmo)*	4.30±0.300	6.2±0.277	4.5±1.5	4.75±1.00	10.00±1.155*	11.00±1.0**

All values expressed as mean ±SD (n=3).

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