

MODULATION OF HUMORAL IMMUNITY BY CASSIA FISTULA AND AMOXY-CASSIA

NAFISA HASSAN ALI, SHAHANA UROOJ KAZMI AND SHAHEEN FAIZI*

Immunology and Infectious Disease Research Laboratory, Department of Microbiology,
University of Karachi, Karachi-75270

*International Center for Chemical Sciences (ICCS), HEJ Research Institute of Chemistry,
Dr. Panjwani Center for molecular Medicine and Drug Research, University of Karachi, Karachi-75270

ABSTRACT

Immunomodulatory effect of fruit of *C. fistula*, a traditional medicinal plant and its synergistic antimicrobial combination with amoxicillin named 'Amoxy-cassia' (Patent # 1371240, Government of Pakistan) studied on humoral immune system of BALB/c mice. Animal immunized with sheep RBC and treated with *C. fistula* fruit, amoxycassia, amoxicillin and saline. Number of activated anti-SRBC producing cell in spleen calculated by haemolytic plaque assay. Antibody titer in blood was measured by haemagglutination test. Number of plaques formed by the animal treated with Amoxy-cassia, amoxicillin, *C. fistula*, and normal saline were 191, 86, 53, 34 per 10^5 spleen cells respectively. Haemagglutinating Antibody (HA) titer was evaluated on post-immunized day 4, 6, 8, 10. Rising antibody titer was observed in all animals but Amoxy-cassia treated mice serum had the highest HA titer through out the experiment suggesting its therapeutic usefulness. But further detailed studies of mechanisms of immunomodulation and its probable use in immuno-compromised individual are still to be investigated and their possible use as an adjuvant during vaccination programs in order to reduce number of non-responder to vaccines need to be studied.

Keywords: *Cassia fistula*, immunomodulator, medicinal plant, humoral immunity, amoxicillin.

INTRODUCTION

The plant products have long been used as immunomodulators by the traditional healers (Tan *et al.*, 2004). Immunostimulants enhance the overall immunity of the host, and present a non-specific response against the microbial pathogens. They also work to heighten humoral and cellular immune responses, by either enhancing cytokine secretions, or by directly stimulating B or T lymphocytes. In clinical medicine they are used to treat patients suffering from AIDS, autoimmune disease and to prevent graft rejection (Puri, *et al.*, 2000; Zhang, *et al.*, 1995). It is reported that 64% of the world population use botanic drug to combat health problems. Currently it is estimated that almost 50% of the synthetic medicines are derived from, or patterned after phytochemicals.

In present study effect of *C. fistula* fruit, amoxy-cassia, and amoxicillin (Ali, *et al.*, 2007) on the humoral defence mechanism of BALB/c mice was evaluated. *C. fistula* (golden shower) is the medicinal plant used to treat malaria, black water fever, blood poisoning, dysentery, round worm infection, facial paralysis and rheumatism (Jayaweera, 1981; Bhakata *et al.*, 1998; Bhavan, 1992; Misra, 1981). This is the first report of *C. fistula* fruit showing immunomodulatory effect. Amoxy-cassia a novel, synergistic anti-microbial combination that was *in vitro* found effective against multi-drug resistant *Salmonella enterica* serovar Typhi (Ali *et al.*, 2007;

Labadie *et al.*, 1989). Amoxicillin is a beta lactam antibiotic (Maiti *et al.*, 1998).

MATERIALS AND METHODS

Plant Collection and Authentication

Fruit of *C. fistula* plucked from the trees growing at Department of Microbiology, University of Karachi, Karachi, Pakistan in the month of July. Taxonomist Mr. Jan Alam authenticated the plant. A voucher specimen bearing general herbarium number 68508 deposited in herbarium of Karachi University.

Preparation of the stock solutions

C. fistula: Fruits were first shade dried, grounded to powder form, 3-mg powder was dissolved in 100 ml of the sterile distilled water, boiled for two minutes with continuous stirring, cooled and left undisturbed for two minutes, the procedure was repeated twice. Extract was filtered under sterile conditions and the filtrate stored at 0°C. Aqueous solution of amoxicillin (3mg/ml) was prepared in sterile distilled water.

Animals used

Six weeks old, BALB/c mice of either sex weighing 20-25 gm obtained from animal house of HEJ Research Institute of Chemistry. The animals maintained on standard pellet diet and water *ad libitum* during the entire trial period.

Corresponding author: Email: nafisa@jmc.edu.pk, nafeesa29@yahoo.com

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Antigen sheep erythrocytes (SRBC)

Sheep RBC washed thrice with pyrogen free sterile normal saline and divided into two parts, first part adjusted to a concentration of 5×10^6 cells/ml for immunization. While second portion was kept in alsever's medium and stored at 4°C.

Treatment of BALB/ C mice

In this experiment 32 BALB/c mice of either sex were divided into 4 groups each group contained eight mice. On day zero, first group of mice injected intra-peritoneally with 10 µg/ml of Amoxy- cassia. Second group treated with 10 µg/ml of *Cassia fistula*. Third group treated with 1 µg/ml of amoxicillin. Group 4 animals treated with saline. On day '1' same treatment as day '0' was given to each animal and then 0.2 ml of 10% washed SRBC were injected in the peritoneum of all the experimental animals. On day 2, group one animal injected intra peritoneally with 5 µg of amoxy-cassia. Group 2 mice received 5 µg/ml of *C fistula* fruit solution. Animals in group-3 treated with 0.5 µg/ml of amoxicillin introduced. Group 4 animals received normal saline.

Collection of blood

Mice anaesthetized with ether and bled from the retro orbital plexus by means of capillary tubes and serum separated by centrifugation.

Anti- SRBC antibody titer by haemagglutination (H.A.)

Blood from the treated and control mice were collected on day 4, 6, 8 and 10-post immunization. Two fold serial dilutions of serum from each group of mice prepared in micro-titer plate. 20 µl of 1% sheep RBC added in each well and thoroughly mixed. Plates incubated at 37°C for two hrs, wells observed for matrix /button formation. Hemagglutination titer (H.A) is the reciprocal of the highest dilution showing matrix formation (Talwar, 1983).

Hemolytic plaque assay for detecting anti-SRBC antibody secreting plasma cells

The Spleen cells of SRBC immunized treated mice were separated in RPMI-1640 medium (Biochrom Wetheim Germany). Washed twice and suspended in the same medium at cell density 10^5 spleen cells/ml. Glass slides were layered with 0.1% agarose in hank balanced salt solution. The suspension containing 100 µl/ml of spleen solution and 10% RBC are mixed with plaquing medium (1% agarose in hank balance salt solution). Mixture poured on to the pre-coated glass slides (0.1% agarose coated). Slides incubated at 37°C for one hour. Then 8 ml of 1:2 diluted guinea pig serum in veronal saline was poured on the slide and incubated for 30 minutes at 37°C. Number of plaques/ 10^5 cells evaluated. To confirm the results slides incubated at 4°C over night and plaque counted again (Talwar, 1983).

Statistical analysis

All experiment were performed were performed in triplicate and the results were expressed as mean $\pm$ S D for three experiments. P values in a two tailed student test found to be < 0.05 .

RESULT

Haemolytic plaque assay

The result shows that the number of anti-sheep RBC secreting cell activated in the spleen of amoxy-cassia treated BALB/c mice was highest (fig. 1).

Haemagglutination titer

Increasing titer of haemagglutinating antibody was observed on post immunization day 4, 6, 8, 10 in treated animals (fig. 2). However significant increase in HA titer was observed in amoxy-cassia and *C. fistula* (alone) treated mice.

DISCUSSION

Spleen is the major secondary lymphoid organ that responds to systemic infection and contributes to both humoral as well as cellular arm of immune system. Being the part of humoral system, in presence of antigen it activates cells that secrete antibodies, which are present in its lymphoid tissue (Tiwari, *et al.*, 2004). In present study we have evaluated the number of activated antibody producing cell against sheep RBC that served as specific antigen in treated and untreated BALB/c.

In vitro when antigen-antibodies form complex complement is fixed, when additional complement obtained from rabbit or guinea pig is added in the system, it results in the formation of hemolytic plaques with an antibody-producing cell in its center. It was therefore possible to estimate the number of specific antibody secreting cells in a given lymphoid cell suspension of spleen. It is assumed that direct plaque are produced by IgM type antibodies producing cells because IgM binds with higher efficiency to complement and the fact that rise in the number of direct plaques after primary immunization occurs on fourth day in parallel with the high titer of specific IgM antibodies in serum. At neutral pH, red blood cells possess negative ion cloud that makes the cell repel from one another, this repulsive force is referred as zeta potential. Because of its size and pentameric nature IgM can overcome the electric barrier and get cross-link red blood cells, leading to subsequent agglutination. The smaller size and bivalency of IgG however makes them less capable to overcome the electric barrier. This characteristic may account for IgM being more effective than IgG in agglutinating red blood cells. *C. fistula* and amoxy-cassia treatment improved the haemagglutination antibody titer in blood collected before and after treating the immunized animal and the rising titer in number of

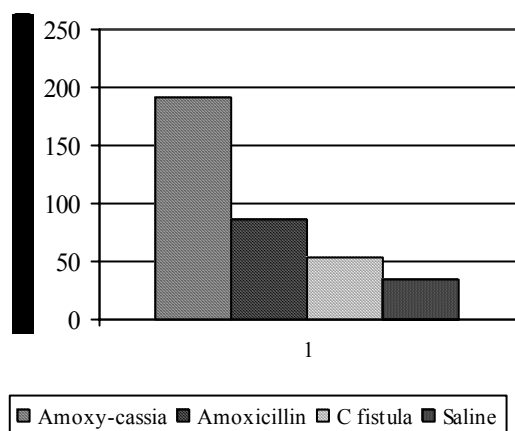


Fig. 1: Influence of amoxy-cassia, amoxicillin and aqueous fruit extract of *C. fistula* on humoral immune system was studied by evaluating the haemagglutination titer of BALB.c mice immunized with sheep RBC and treated with different substances and the number of anti SRBC producing cell activated in the spleen was counted as plaque formed per 10^5 spleen cells. Results are given as an average standard $\pm$ for three experiments. P value in a two tailed student 't' test with HA titer of the test with control was < 0.05 .

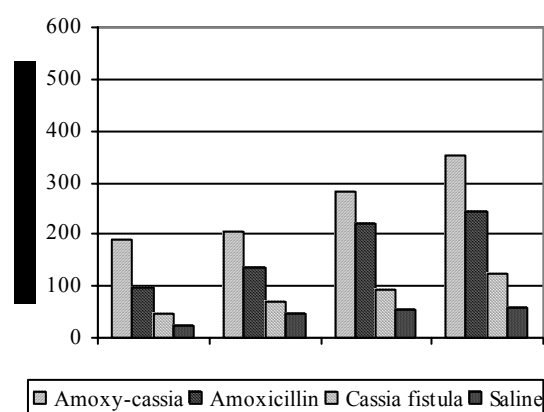


Fig. 2: Influence of amoxycassia, amoxicillin and aqueous fruit extract of *C. fistula* on humoral immune system was studied by evaluating the haemagglutination titer of BALB.c mice immunized with sheep RBC and treated with different compounds. Blood sample were collected on days indicated. Results are given as an average standard $\pm$ for three experiments. P value in a two tailed student t test with HA titer of the test with control was < 0.05 .

specific antibody reflected an overall elevation of humoral immune response.

CONCLUSION

The present study shows that amoxy-cassia (Patent # 137124) and water extract of fruit *C. fistula* stimulates immune system by activating large number of anti RBC producing cells in the spleen that suggest its therapeutic usefulness. But further detailed studies of mechanisms of immuno-modulation and probable use in immuno compromised individual are still to be investigated and their possible use as an adjuvant during vaccination programs in order to reduce number of non- responder to vaccines need to be studied.

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