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Research Article

STUDIES ON IMMUNOMODULATORY ACTIVITY OF *FICUS CARICA*

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ABSTRACT

The Immunomodulatory effect of ethanolic extract of the leaves of *Ficus carica* (Moraceae) was investigated in mice. The study was carried out by various hematological and serological tests. Administration of extract remarkably ameliorated both cellular and humoral antibody response. It is concluded that the test extract possessed promising immunostimulant properties.

Keywords: *Ficus carica*; Immunomodulatory activity; leaves; Hematological and Serological tests.

INTRODUCTION

The medicinal properties of certain plants have been known for centuries[1]. More than a quarter of the medicines in use today come from plants, i.e. from traditional medicine. Currently, with the active encouragement of the WHO[2,3].

The immune system is known to be involved in the etiology as well as pathophysiologic mechanism of many diseases. Immunology is thus probably one of the most rapidly developing areas of biomedical research and has great promises with regard to prevention and treatment of wide range of disorders, inflammatory diseases of skin, gut, respiratory tract, joints and central organs. In addition infectious diseases are now primarily considered immunological disorders while neoplastic diseases and organ transplantation and several autoimmune diseases may involve in an immunosuppressive state[4].

Modulation of immune response to alleviate the disease has been a interest for many years and the concept of *Rasayana* in Ayurveda is based on related principles. The function and efficacy of immune system may be influenced by many exogenous factors like food and pharmaceuticals, physical and psychological stress and hormones etc. resulting in either immunostimulation or immunosupression. The healthy state is believed to be based on a sophisticated fine tuning of immonoregulatory mechanisms.

Suppressive and cytotoxic activity affecting the function of immune system has been reported for many of the synthetic substance, azathioprin and cyclophosphamide are bused but these drugs have a number of side effects in long term treatment. Cyclophosphamide is an alkylating agent resulting in the cross linking of DNA and causes inhibition of DNA synthesis. The major drawback of these drugs is myelosuppression, which is undesirable[5].

Figs (*Ficus carica* L.) are a widespread species commonly grown, especially in warm, dry climates. The ideal condition for intensive cultivation of figs is a semi-arid climate with irrigation. The world production of figs is about one million tons, and it is mostly concentrated in the Mediterranean. In this area, figs have been grown for centuries and are the most frequently mentioned fruit in the Bible[6].

Ficus carica Linn. (Syn: *Ficus sycomorous*; Family: Moraceae) is commonly referred as "Fig". Its fruit, root and leaves are used in the native system of medicine in different disorders such as gastrointestinal (colic, indigestion, loss of appetite and diarrhea), respiratory (sore throats, coughs and bronchial problems), inflammatory and cardiovascular disorders[7,8]. Fig has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedy[9, 10].

The root is tonic, useful in leucoderma and ringworm. The fruit is sweet, antipyretic, tonic, purgative useful in inflammation, weakness, paralysis, thirst "*Vatta* diseases" of head, diseases of liver and spleen, pain in chest, cures piles, stimulate growth of hair. The milky juice is expectorant, diuretic, and dangerous for eye. Fig latex is used as an anthelmintic[11].The *Ficus carica* leaf has been reported hypoglycaemic[12], hepatoprotective[13] and latex reported the anthelmintic[14]activity.

Plant derived natural products such as flavonoids, terpenoids and steroids etc.have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and Immunomodulatory activity[15-17]. Hence the claim it was thought worthwhile to study the Immunomodulatory activity of *Ficus carica* leaf.

MATERIALS AND METHODS

Plant material

The leaves of *Ficus carica* Linn. (Moraceae) were collected from Pal in the Khandesh region of the Jalgaon district of Maharashtra (India) in Auguest 2008**.** A voucher specimen was deposited in the Central National Herbarium, Botanical Survey of India, Pune, Maharashtra dated 13/08/2008 (BSI/WC/Tech/2008/355) and was authenticated by P. G. Diwakar (Joint Director). The leaves were collected and dried under shade and pulverized in a mechanical grinder and stored in closed container for further use.

Preparation of extract

The powdered leaves (1kg) was packed and defatted with petroleum ether (60-70^{0}C.about 25-30 cycles) in a soxhlet extractor. The defatted material was subjected to ethanolic extraction using 95% ethanol in soxhlet extractor (40cycles) the extract was filtered through whatman filter paper.

Test animals

Healthy mice (25-30 g) of either sex were selected for the study. The animals were fed on commercial diet (Hindustan lever pellets, Bangalore) and water ad *libitum* They were acclimated to laboratory hygienic condition for ten days before starting the experiment. The experimental protocol and animal house has been approved by the institutional animal ethics committee and by the animal regulatory body of the Indian Government (Registration No.652/02/a/ CPCSEA, dated 25/ 01/1999).

Administration of test extract

The method of pyrogallol induced immunosupression[18] was employed with slight modification to study the Immunomodulatory potential of the extract. Animals were randomly divided in to four groups, consisting of six animals each. Group I animals were administered pyrogallol (100mg/kg/ipdaily for seven days). Group II animals served as control and received equivalent volume of sodium CMC (0.1%w/v) as a vehicle. Group III animals were given pyrogallol daily for seven days with the same dose and the test

extract, suspended in 0.1% (w/v) Sodium Carboxymethyl Cellulose (CMC) with 100 mg/kg daily, p.o. from day 8 to 22. Group IV animals were given with pyrogallol daily for seven days and vitamin E suspension (150mg/kg p.o.) beside above treatment, all the groups received sheep Red Blood Cells (SRBC, 0.5 × 109cells/100g, i.p.) on day 7 and 13, as the antigenic material to sensitize them for immunological studies.

Humoral antibody response to SRBC

Measurement of antibody titer by haemaglutination reaction was done by the method of Miller et al[19].

On 13th and 20th day, blood samples were collected from the retro orbital plexus and the mice serum was used for determination of haemaglutination titer. The blood samples were centrifuged to collect serum and equal volumes of individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 25µl normal saline in microtitration plates was added 25µl of 1% SRBC suspension in saline. After mixing, the plates were incubated at 37^0 C for 1 hr and examined for haemaglutination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titer.

Cellular immune response (foot pad reaction test)

To study the cellular immune response, the edema was induced in the right paw of mice by injecting SRBC (0.025×10^9cells) in the sub planar region on 20th day, the increase in paw volume in 48 h, i.e. on 22nd day was assessed on digital plethysmometer (UGO basile-7150) the mean percentage increase in foot pad volume was considered as delayed type hypersensitivity and as an index of cell mediated immunity. The volume of the left hind paw, injected similarly with phosphate buffered saline served as a control.

RESULTS AND DISCUSSION

Pyrogallol induced suppression of humoral as well as cell mediated immune response were significantly attenuated by daily oral treatment with ethanolic extract of *Ficus carica* leaves. Vitamin E treated group exhibited similar attenuation of the suppression in immune responses. *Ficus carica* leaves ethanolic extract at the dose of 100mg/kg was found to suppress delayed time hypersensitivity reaction induced by SRBCs in mice (Table II)

It reveals effect of drug on T-lymphocytes and other cell types required for expression of humoral response to SRBCs, as evidenced by marked increase in haemaglutination titres in mice was also observed (Table I)

In conclusion, the results obtained in the present study show that *Ficus carica* leaves ethanolic extract produces stimulatory effect on the humoral and cell mediated immune response in the experimental animals and suggest its therapeutic usefulness in disorders of immunological origin. Further studies using *in vivo* and *in vitro* models of immunomodulation are needed to confirm the Immunomodulatory activity of *Ficus carica* leaves and its mechanism of action.

Table 1: Influence of ethanolic extract of *Ficus carica* leaves and vitamin E on the primary and secondary humoral immune responses to sheep RBC

Groups	Mean antibody titer	
	Primary	Secondary
Control group (Normal saline)	8.6±0.23	11.3±0.17
Immunosuppressed (Pyrogallol treated)	6.3±0.16	8.4±0.20
Experimental(100mg/kg ethanolic extract of *Ficus carica* leaves)	7.5±0.25	10.36±0.27
Standard (Vitamin E suspension,150.0mg/kg)	8.36±0.54	11.55±0.82

(n=6) ± Standard deviation, p<0.05, when compared with control group in all cases (statistics; one way ANOVA followed by Dunnets't test)

Table 2: Influence of ethanolic extract of *Ficus carica* leaves and vitamin E on cell mediated immune responses to sheep RBC

Groups	Mean increase in paw volume
Control group (Normal saline)	32.38±0.47
Immunosuppressed (Pyrogallol treated)	16.27±0.26
Experimental(100mg/kg ethanolic extract of *Ficus carica* leaves)	25.40±0.48
Standard (Vitamin E suspension,150.0mg/kg)	20.75±2.45

(n=6) ± Standard deviation, p<0.05, when compared with control group in all cases (statistics; one way ANOVA followed by Dunnets't test)

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