



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH

An official Publication of Human Journals

ISSN 2349-7203



Human Journals

Research Article

May 2015 Vol.:3, Issue:2

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## Evaluation of Immunomodulatory Activity of Ethanolic Extract of Stem Bark of *Coscinium fenestratum* (Gaertn.) Colebr



IJPPR  
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals



ISSN 2349-7203

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**Submission:** 9 May 2015  
**Accepted:** 14 May 2015  
**Published:** 25 May 2015

**Keywords:** *Coscinium fenestratum* (Gaertn.) Colebr, antibody titer, rasayana, HA, DTH

### ABSTRACT

*Coscinium fenestratum* (Gaertn) Colebr, commonly known as Tree turmeric, false calumba, Daru Haridra has been used as Rasayana drug. Ayurvedic literature states that rasayana means rejuvenating, vitalizer and immunomodulator. The assessment of immunomodulatory activity of ethanolic extract (Crude extract), alkaloid fraction and non-alkaloid fraction of stem bark of *Coscinium fenestratum* was carried out by performing Hemagglutinating antibody titer (H.A.) and delayed type of hypersensitivity. Ethanolic extract of stem bark of *Coscinium fenestratum* (CF) was administered orally at doses of 40, 80, 120 mg/kg, alkaloid fraction (CFA) administered orally at doses of 1, 2, 4 mg/kg and non-alkaloid fraction (CFNA) administered orally at doses of 40, 80, 120 mg/kg to healthy mice and antibody response was evaluated at the seventh day of treatment. The alkaloid fraction administration produced a significant fall in H.A. titer as compared to control group. There was significant mean difference in the footpad thickness in alkaloid fraction group when compared with control group. Cyclophosphamide was used as immunosuppressant and Immunocin was used as Immunostimulant. Sheep red blood cells were used as an antigen. This investigation indicates that *Coscinium fenestratum* has significant immunomodulatory activity.



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## INTRODUCTION

*Coscinium fenestratum* (Gaertn.) Colebr (Menispermaceae), commonly known as, 'tree turmeric', is widely distributed in Western Ghats (Tamilnadu and Kerala, India) and Ceylon. It is a woody climbing shrub with cylindrical stem, externally yellowish-brown and internally yellow and longitudinally fluted<sup>1</sup>.

The stem is bitter, thermogenic, ophthalmic, anodyne, anti-inflammatory, vulnerary depurative, stomachic, antiseptic, febrifuge, sudorific, tonic and useful in vitiated conditions of kapha and vata<sup>2</sup>. The plant is traditionally used in ophthalmopathy, inflammation, wounds, ulcers, skin diseases, abdominal disorders, jaundice, diabetes, fever and general debility<sup>3,4</sup>.

Previous studies have reported that the alcoholic extract of this plant possesses anti-oxidant activity, it could protect against hepatotoxicity<sup>5</sup>, hypotensive action<sup>6</sup>, anti-diabetic action<sup>7</sup>, the stem-bark contains berberine, ceryl alcohol, sitosterol, palmitic acid, oleic acid, and saponin<sup>8</sup>.

The present investigation was aimed to study the immunomodulatory activity of ethanolic extract of stem bark of *Coscinium fenestratum* (Gaertn.) Colebr (CF) using reported methods in order to justify the traditional claims endowed upon the drug as rasayana.

## MATERIALS AND METHODS

### Plant material

The stem-barks of the plant *Coscinium fenestratum* were purchased from the Nagarjuna Herbal Concentrates Ltd. Alakkode, Kerala. The plant was identified and authenticated from Agharkar Research Institute (An Autonomous Institute of Department of Science and Technology, Govt. of India) G. G. Agharkar road, Pune - 411004, India, on 26/10/2006.

### Experimental animal

All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the purpose of control and supervision on experimentation on animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee.

Swiss albino mice weighing between 18 to 25 gm of either sex were used. The above animals of either sex were purchased from National Toxicology Center, Pune. Animals had free access to standard pellet diet and water *ad libitum*. Fresh animals were used for each experiment.

### **Drugs and chemicals**

Cyclophosphamide 50 mg/kg was used as a standard immunosuppressant. Imunocin 30 mg/kg was used as Immunostimulant. Sheep red blood cells (SRBCs) were washed three times with normal saline and adjusted to the required concentration for immunization and for challenge as antigen.

### **Humoral immune response**

#### **Hemagglutinating antibody (H.A.) titer:**

#### **Sheep RBC (SRBC)**

Sheep blood was collected from local slaughterhouse in sterile 0.49 % EDTA solution in 1:1 proportion of EDTA in saline (freshly prepared). Blood was kept in the refrigerator and processed for the preparation of Sheep RBC by centrifuging at 2000 rpm for 10 minutes and washing with physiological saline 4-5 times<sup>8</sup>.

#### **Antigen Challenge**

On 1<sup>st</sup> day and 7<sup>th</sup> day of the study, mice from all the groups (i.e. group I to XII) were immunized and challenged respectively, with sheep RBC's (20%) in normal saline intraperitoneally<sup>9,10</sup>.

#### **Procedure**

Animals were divided into 12 groups of six animals each. Animals in group I received orally 1ml D.W. for 7 days. Group II received daily dose of Imunocin 30 mg/kg/d (oral). Group III received daily dose of Cyclophosphamide 50 mg/kg (p.o.) only on day 4, 5, 6. Animals in group CFI, CFII and CFIII were administered crude extract at doses of 40, 80 and 120 mg/kg/day, p.o. respectively for 7 days. Animals in group CFNAI, CFNAII and CFNAIII were administered Non-alkaloid fraction at doses of 40, 80 and 120 mg/kg/day, p.o. respectively for 7 days. Animals in group CFAI, CFAII and CFAIII were administered alkaloid fraction at doses of 1, 2 and 4 mg/kg/day p.o. for 7 days. The animals were immunized by injecting 0.1 ml of 20% of fresh sheep red blood cells suspension intraperitoneally on day 0<sup>9,11</sup>.

Blood was withdrawn on 7<sup>th</sup> day from retro-orbital plexus from all antigenically sensitized mice respectively. Blood was centrifuged to obtain serum, normal saline was used as a diluents and SRBC was adjusted to (20%). Each well of a micro titer plate was filled initially with 25 µl of saline. 25 µl of serum was mixed with 25 µl of saline in the first well of microtiter plate. Subsequently the 25 µl diluted serum was removed from first well and added to the next well to get two fold dilutions of the antibodies present in the serum. Further two fold dilutions of this diluted serum were similarly carried out till the last well of the second row (24<sup>th</sup> well), so that the antibody concentration of any of the dilutions is half of the previous dilution. 25 µl SRBC (20%) were added to each of these dilutions and the plates were incubated at 37°C for one hour and then observed for hemagglutination. The highest dilution giving hemagglutination was taken as the antibody titer. The antibody titers were expressed in the graded manner, the minimum dilution (1/2) being ranked as 1, and mean ranks of different groups were compared for statistical significance<sup>11</sup>. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titer.

### **Cell mediated immune response**

#### **Delayed Type Hypersensitivity (DTH)**

Animals were divided into 12 groups of six animals each. Animals in group I received orally 1ml D.W. for 7 days. Group II received daily dose of Imunocin 30 mg/kg/d (p.o.). Group III received daily dose of Cyclophosphamide 50 mg/kg (p.o.) only on day 4, 5, 6. Animals in group CFI, CFII and CFIII were administered crude extract at doses of 40, 80 and 120 mg/kg/day, p.o. respectively for 7 days. Animals in group CFNAI, CFNAII and CFNAIII were administered non-alkaloid fraction at doses of 40, 80 and 120 mg/kg/day, p.o. respectively for 7 days. Animals in group CFAI, CFAII and CFAIII were administered non-alkaloid fraction at doses of 1, 2 and 4 mg/kg/day, p.o. For 7 days. Mice from all the groups were immunized with 0.1 ml of 20% SRBC's in normal saline intraperitoneally on 0 day of the study. On day 7<sup>th</sup> of the study, animals from all the group were challenged with 0.03 ml of 1% SRBC's in subplantar region of right hind paws. Foot pads reaction was assessed after 24 hr. i.e. on 8<sup>th</sup> day, in terms of increase in the thickness of footpads due to oedema caused as a result of hypersensitivity reaction. Odema was measured with digital vernier calliper. The footpad reaction was expressed as the difference

in the thickness (mm) between the right foot pad injected with SRBC and the left footpad injected with normal saline<sup>9,12</sup>.

### **Statistical analysis**

The results are expressed as mean  $\pm$  S.E.M. Data was analyzed by one way ANOVA followed by Tukey-Kramer multiple comparison test. Value of  $p$  less than 5% (i.e.  $p < 0.05$ ) was considered statistically significant.

## **RESULTS AND DISCUSSION**

### **Effect of *Coscinium fenestratum* on Humoral Immune Response**

At the end of 7<sup>th</sup> day, the animals of each group were determined for antibody titer as shown in the Table No. 1

Mice in the Cyclophosphamide control group had shown significantly less H.A. titer (\*\* $p < 0.01$ ) was compared to control.

CFA II and CFA III administration produced a significant fall in H. A. Titer (\*\* $p < 0.01$  and \*\*\* $p < 0.001$  respectively) was compared with control group. Imunocin, 30 mg/kg (p.o.) showed a significant rise in H. A. Titer (\*\* $p < 0.001$ ) was compared with control group.

### **Effect of *Coscinium fenestratum* on Cell mediated immune response**

In Delayed Type Hypersensitivity, the degree of Footpad oedema was measured after 24 hours. The DTH skin response requires antigen specific memory T cells and produces inflammation. The inflammation results from the production of local cytokines and chemotaxis at the site of injection, which results in the recruitment of large number of neutrophils and mononuclear cells.

The result shown in Table No.2 indicates that cyclophosphamide group has shown significant decrease, (\*\* $p < 0.001$ ) in the mean difference, in the paw thickness as compared to control group.

There was no significant decrease in the mean difference, in the footpad thickness in all groups except Cyclophosphamide (\*\* $p < 0.001$ ), CFA II (\*\* $p < 0.01$ ) and CFA III (\*\* $p < 0.001$ ) administered group when compared with control group. While Imunocin treated group showed

significant rise (\*\*\*) $p < 0.001$ ) in the mean difference in footpad thickness when compared with control group.

The humoral immunity involves interaction of Beta cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters and they are more readily ingested by phagocytic cells. To evaluate the effect of *C. fenestratum* on humoral response, its influence was tested on sheep erythrocyte specific haemagglutination antibody titre in mice. Cyclophosphamide at a dose of 50 mg/kg, p.o. showed significant inhibition in antibody titre response. Imunosin showed significant increase in antibody titre response at a dose of 30 mg/kg p.o. The anti-SRBC antibody titer in alkaloidal groups has decreased antibody titre in dose increasing manner at alkaloidal doses 2mg/kg as well as higher dose 4mg/kg. But treated doses of crude extract and non-alkaloid group did not show any significant increase or decrease in antibody titer. This indicates the decrease in responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis<sup>13</sup>.

DTH is a part of the process of graft rejection, tumour immunity, immunity to many intracellular infectious microorganisms especially those causing chronic diseases such as tuberculosis<sup>13</sup>. DTH requires the specific recognition of a given antigen by activated T lymphocytes which subsequently proliferate and release cytokines. These in turn increase vascular permeability, induce vasodilatation and macrophage accumulation<sup>14</sup>.

In the model of delayed type hypersensitivity, the groups of mice with normal immune status treated with ethanol extract 40, 80 and 120mg/kg was not potentiated when challenged with SRBC as compared to control group. Alkaloidal group treated with 2mg/kg showed moderate difference in footpad thickness. Significant decrease in DTH response is showed by alkaloidal group treated with 4mg/kg.

In the immunosuppressed groups Cyclophosphamide was used as immunosuppressant as it elevates the DTH response. It has been established that the mechanism behind this potentiation of DTH by cyclophosphamide is the elimination of population of suppressor cell.

Imunocin was potentiated when challenged with SRBC as compared to control group but it was found statistically significant. This study thus demonstrates that the plant *Coscinium fenestratum* possesses the Immunosuppressant activity. It may be because of presence of alkaloids such as Berberine and other derivatives of berberine.

**Table 1. Hemagglutinating antibody (H.A.) titer:**

Effect of *Coscinium fenestratum* on Hemagglutinating antibody (H.A.) titer

Sr. no.	Groups (n=6)	Antibody Titer
1	Control	7.2 ± 0.37
2	Imunocin	10.4 ± 0.50 ***
3	Cyclophosphamide	3.4 ± 0.20***
4	CF I	6.6 ± 0.50
5	CF II	8.0 ± 0.54
6	CF III	7.2 ± 0.37
7	CFNA I	7.0 ± 0.37
8	CFNA II	7.6 ± 0.40
9	CFNA III	8.0 ± 0.50
10	CFA I	7.0 ± 0.45
11	CFA II	5.0 ± 0.44**
12	CFA III	4.6 ± 0.24***

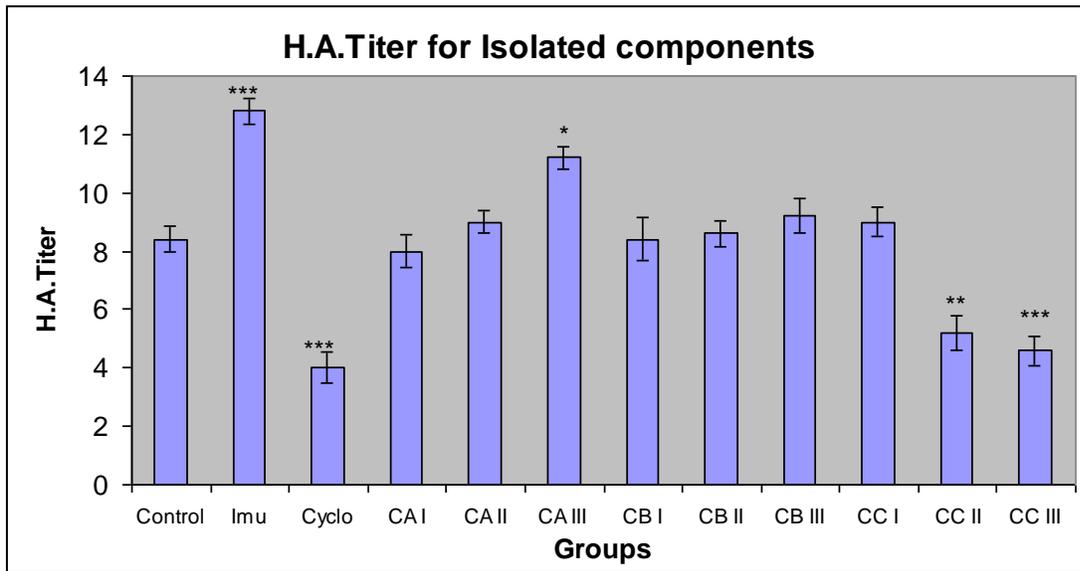
Values are expressed as Mean ± S.E.M.

\*\* = p<0.01 and \*\*\* = p<0.001

Test drug, Imunocin and Cyclophosphamide treated groups were compared with Control Group.

(Statistically analysed by one way ANOVA followed by Tukey-Kramer multiple comparisons test).

Fig. No. 1



CA- COMPONENT A

CB- COMPONENT B

CC- COMPONENT C

Values are expressed as Mean ± S.E.M.

\*= $p < 0.05$ , \*\* =  $p < 0.01$  and \*\*\* =  $p < 0.001$

Test isolated components treated groups, Imunocin treated group and Cyclophosphamide group were compared with control group.

(Statistically analysed by one way ANOVA followed by Tukey-Kramer multiple comparisons test.)

**Tabel 2. Cell mediated immune response**

Effect of *Coscinium fenestratum* on cell mediated immune response by delayed type hypersensitivity induced footpad oedema.

Sr. no.	Groups (n=6)	Footpad thickness (Mean Difference) (mm)
1	Control	1.011 ± 0044
2	Imunocin	1.084 ± 0.041***
3	Cyclophosphamide	0.72 ± 0.03***
4	CF I	1.012 ± 0.05
5	CF II	1.012 ± 0.045
6	CF III	1.013 ± 0.05
7	CFNA I	1.014 ± 0.03
8	CFNA II	1.015 ± 0.04
9	CFNA III	1.014 ± 0.042
10	CFA I	1.011 ± 0.055
11	CFA II	0.988 ± 0.045**
12	CFA III	0.898 ± 0.035***

CF: ethanolic extract of *C. fenestratum*

CFNA: non alkaloid fraction of *C. fenestratum*

CFA: alkaloid fraction of *C. fenestratum*

Values are expressed as Mean ± S.E.M.

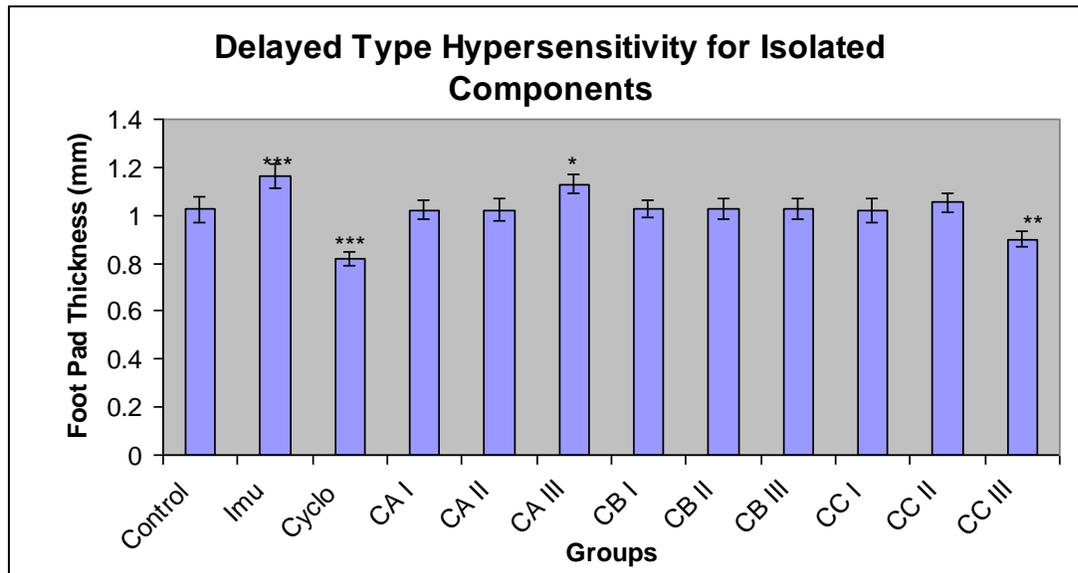
\*\* = p<0.01 and \*\*\* = p<0.001

Test drug and Imunocin treated groups were compared with control group.

Cyclophosphamide group was compared with control group.

(Statistically analysed by one way ANOVA followed by Tukey-Kramer multiple comparisons test.)

Fig. No. 2



CA- COMPONENT A

CB- COMPONENT B

CC- COMPONENT C

Values are expressed as Mean  $\pm$  S.E.M.

\*= $p < 0.05$ , \*\* =  $p < 0.01$  and \*\*\* =  $p < 0.001$

Test drug and Imunocin treated groups were compared with control group.

Cyclophosphamide group was compared with control group.

(Statistically analysed by one way ANOVA followed by Tukey-Kramer multiple comparisons test.)

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