Research Article

Evaluation of Immunomodulatory Activity of *Cassia fistula*

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ABSTRACT

To investigate the immunomodulatory effect of Cassia fistula in rats. *Cassia fistula* was administered orally at doses of 100 and 200mg/kg to healthy rats divided into five groups consisting of six animals each. The assessment of immunomodulatory activity was carried out by testing the humoral (antibody titre) and cellular (foot pad swelling) immune, responses to the antigenic challenge by sheep RBCs and by neutrophil adhesion test. On oral administration of the extract, a significant increase in neutrophil adhesion and delayed type hypersensitivity response whereas the humoral response to sheep RBCs was unaffected. Thus Cassia fistula significantly potentiated the cellular immunity by facilitating the foot pad thickness responses to the sheep RBCs in sensitized rats with a dose of 100 and 200mg/kg the DTH response (mean + S.D. % increase in paw volume). The responses were statistically significant when they were compared with the control. The study stated that *Cassia fistula* shows a significant stimulation of the cell mediated immunity and no effects on the humoral immunity.

Keywords: Cassia fistula, immunomodulatory activity.

INTRODUCTION

Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases[1]. Thus the present investigation was aimed at evaluating the immunomodulatory activity of Cassia fistula (Linn.) leaves on standard animal models. Cassia fistula (Linn.) belonging to the family is fabaceae, soft perennial plant abundantly found in Northern Western Himalayas. It is generally known as golden shower. There are many medicinal uses for C. fistula known from Asia. The flesh of the fruit is used as laxative, while the bark can be used to treat skin infections. In India the strongly scented pulp is sometimes added to tobacco and smoked.

Experimental

MATERIALS AND METHODS

Plant Material

Fresh leaves of the plant Cassia fistula (Linn.) were obtained and identified from authentic sources. A voucher specimen has been identified and deposited at the Department of Botany Shri Shivaji Sr. College, Chikhli Dist. Buldana. The collected leaves were dried in shade, crushed to coarse powder and used for further studies.

Preparation of Extract

The dried plant material leaves (1 kg) were subjected to continuous hot extraction with petroleum ether and ethanol for 36 hours. After extraction with petroleum ether and ethanol. The extract was filtered, concentrated and the solvent was removed by rotary evaporator. The extract was dried over a dessicator. The residue was used for this study. The extracts were subjected to preliminary qualitative tests to identify the various phytoconstituents present in leaves [7]. It was observed that petroleum ether extract contained steroids whereas alcoholic contain flavonoids, steroidal saponins, tannins, phenolic substances and carbohydrates.

Antigen

Sheep Red Blood Cells were collected in Alsever's solution, washed three times in large volumes (30 ml) of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5X 109 cells/ml for immunization and challenge.

Animals

Healthy Wistar male rats (100-150 gm) were used for the study. All the animals were housed from animal house which were under standard conditions of temperature (23± 2°C), 12 h light/dark cycles and fed with standard pellet diet and water ad libitum. Fresh sheep red blood cells (SRBC) in Alsever's solution was obtained from authentic sources. The animals were divided into three groups consisting of six animals each. A group of six untreated rats were taken as control (Group I). The extract was fed orally for 07 days at a dose of 100 mg/kg/day (Group II) and 200 mg/kg/day (Group III) for assessment of immunomodulatory effect.

Neutrophil Adhesion Test[8]

On the 14th day drug treatment, blood samples were collected (before challenge) by puncturing the retro orbital plexus into heparanized vials and analyzed for total leucocyte count (TLC) and differential leucocyte count (DLC) by fixing blood smears and staining with Field stain I and II Leishman's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37 °C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below Neutrophil adhesion(%) = NI u-NI t X100 NI u Where NIu= Neutrophil index of untreated blood samples NIt= Neutrophil index of treated blood sample.

Haemagglutinating Antibody (ha) Titre[9]

Rats of group II and III were pretreated with CF for 14 days and each rat was immunized with 0.5X109 SRBC/rat by i.p. route, including control rats. The day of immunization was referred to as day 0. The animlas were treated with CF for 14 more days and blood samples were collected from each rat on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBC (0.025X109 cells). The micro titre plates were incubated at room temperature for 2 hours and examined visually for agglutination. The highest number of dilution of serum showing haem agglutination has been expressed as HA titre.

Delayed Type Hypersensitivity (dth) Response[9]

Six animals per group (Control and treated) were immunized on day 0 by i.p. administration of 0.5X109 SRBC/rat and challenged by subcutaneous administration of 0.025X109 SRBC/ml in to right hind foot pad on day +14. The extract of CF was administered orally from day-14 untill day + 13.DTH responses were measured at 24 h after SRBC challenged on day +14 and expressed as mean percent increase in paw volume (plethysmometrically).

Statistical Analysis

The data was analyzed using one way analysis of variance (ANOVA) followed by Dunnett's Test. P values <0.001 were considered as significant.

Results

CF evoked as a significant increase in neutrophil adhesion (P, 0.001, significance value) at a dose of 100 mg/kg.day in rats. The results of neutrophil adhesion test are shown in Table 1. HA titre did not show any significant increase when CF was orally administered in different dose. The DTH response to SRBC which corresponds to cell mediated immunity showed a dose dependent increase due to treatment with CF. The differences in DTH response were statistically significant which are shown in Table 2.Thus it can be said that CF induced a remarkable enhancement in DTH Response to SRBC in animals.

Table 1: Effect of Cassia fistula on neutrophil adhesion in rats

Groups	Neutrophil index		Neutrophil Adhesion (%)
	UB	FTB	
I (Untreated)	270.80±7.21	226.20±9.20	
II (100 mg/kg, p.o)	290.64±4.21	239.12 ±15.10	15.90
III (200 mg/kg, p.o)	311.44±4.40	241.71±14.12*	59.09

The values are mean + S.D of 6 rats in each group. One way ANOVA followed by Dunnett's test, *p,0.001 Vs group I, UB= untreated blood; FTB=Fiber Treated Blood.

Table 2: Effect of Cassia fistula on HA titre and DTH response to antigenic challenge by sheep RBCs in rats

Groups	HA titre	DTH response (% increase in paw volume)
I (Untreated)	4.74+0.99	7.02+1.84
II (100 mg/kg, p.o)	5.14+0.71	11.53+2.11*
III (200 mg/kg, p.o)	5.39+0.39	15.51+3.39*

The values are mean + S.D. of 6 rats in each group. One way ANOVA followed by Dunnett's test, *p, 0.001 Vs group I,

DISCUSSION

Immunomodulatory agents obtained from plant and animal origin generally enhances the immune responsiveness of an organism against a pathogen by activating the system. In the present investigation CF when administered orally, significant increased in the adhesion of neutrophils to nylon fibers which interrelates to the [process of margination of cells in blood vessels. It was found to be highly significant when compared with control. The HA titre did not show any increase with CF administration. The DTH response directly correlated the
ccell mediated immunity and was found significant. Thus in this process the T-lymphocytes gets sensitized when they are challenged by any antigen which there by gets converted in to lymphoblasts and secrets lymphokines, and attracts the scavenger cells to the site of reaction. The increase in the response indicated that CF has a stimulating effect on the lymphocytes. Thus it can be concluded that alcoholic extract was found to be highly stimulating agent for cell mediated immune responses.

REFERENCES

1. Perianayagam JB, Sharma SK, Joseph A and Christiana AJM. Evaluation of Antidiarrheal Potential of Emblica officinalis. J Ethnopharmacol.2004;95(1): 85-87.
2. Badoni AK. An Ethnobotanical Survey of Pinswari Community – A Preliminary Survey, Bul Bota Survey India. 2000;32:110-115.
3. Rana TS and Datt B. Ethnobotanical Observation Among Jaunsaries of Jaunsar- Bawar, Dehradun (U.P.) India. Intern J Pharmaco. 1997;35: 371-374.
4. Shah NC and Joshi MC. An Ethnobotanical Survey of the Kumaon Region of India. Econo Bot.1971;25:414-422.
5. Singh B, Agarwal PK and Thakur RS. Aesculuside A- A new triterpene glycoside from Aesculus indica., Planta Medica. 1986;52(5):409-410.
6. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, New Delhi, 2005, 110-111.
7. Harbone JB. Phytochemical Methods-A Guide To Modern Techniques of Plant Analysis ,Chapman and Hall London. 1998;42:129,189, 203.
8. Wilkonson PC. Neutrophil Adhesion test: In Vane JK, Ferreria SH, 1st edn, Vol.1 Handbook of Experimental Pharmacology, Springer- Verlag, Berlin. 1978;109.
9. Puri A, Saxena R, Saxena RP and Saxena KC. J Nat Prod. 1933;56:995-999.